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A CURRICULUM ACTIVITIES GUIDE TO WATER POLLUTION AND ENVIRONMENTAL STUDIES. VOLUME 2. APPENDICES

Environmental Protection Agency
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A CURRICULUM ACTIVITIES GUIDE

TO

WATER POLLUTION

and

ENVIRONMENTAL STUDIES:

APPENDICES - Vol-2

U. S. ENVIRONMENTAL PROTECTION AGENCY

OFFICE OF WATER PROGRAMS

MANPOWER DEVELOPMENT STAFF

TRAINING GRANTS BRANCH

1972

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Appendix I Water Quality Parameters

This appendix is included as a technical reference aid to teachers using this guide. It is organized in four parts: (A) Chemistry, (B) Bacteriology, (C) Aquatic Biology, and (D) Engineering and Physics.

A. Chemistry

Chemical parameters are quite specific, can be quantitated relatively quickly and precisely, and can be related to water quality requirements. It is seldom feasible or worthwhile to apply all analytical procedures to a given water sample. However, certain analyses are performed more or less routinely on water samples and are included in this section.

Each part includes an identification of the selected parameter and its common sources. This is followed by a description of the chemistry involved in the more common approaches to the analysis of that parameter. The procedures include references to commercial testing kits and, in some instances, detailed instructions for those who do not have access to commercial kits.

Commercial kits provide effective approaches to rapid and reasonably accurate analyses, especially when time, facilities and lack of trained personnel are limiting factors. Consequently, the procedures include references to the following commercial units:

Delta Model 50 Portable Laboratory, Delta Scientific Corp.,
Lindenhurst, New York 11757

Hach DR-EL Portable Engineer's Laboratory, Hach Chemical Co.,
Ames, Iowa 50010

LaMotte Model #AM-21, LaMotte Chemical Products Co.,
Chestertown, Maryland 21620.

These kits have been identified only because they proved satisfactory during the development of this program. This endorsement does not imply superiority to other units that may be commercially available.

An annotated bibliography appears at the end of this section. The listings include those references which should be readily available when investigating chemical parameters of pollution.

1. Acid-Base Parameters

a. Acidity

Acidity, a measure of the ability of a water sample to neutralize hydroxide (OH^-) ions, is subdivided into free (mineral), un-ionized (weak acid) and total forms. The chemical species which neutralizes the hydroxide ion is identified as the hydrogen (H^+) ion and is present in all water samples.

Some of the substances which contribute to acidity (i.e., serve as sources of hydrogen ions) are depicted in Fig. 1. Direct hydrogen ion donors are depicted within the circle while those outside the circle provide hydrogen ions directly.

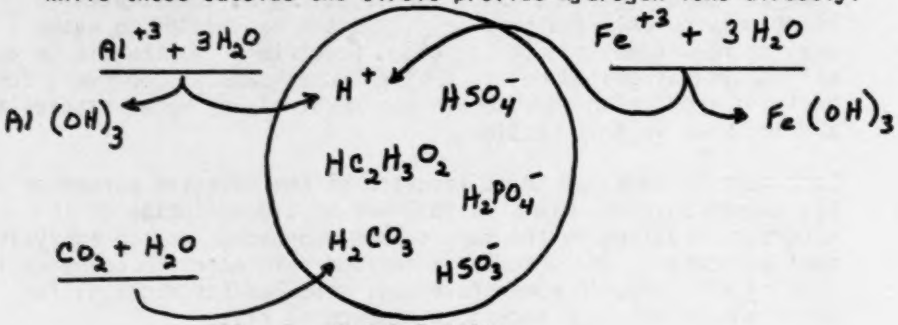

$Al^{+3} + 3H_2O$
$Al(OH)_3$
H^+
HSO_4^-
$Fe^{+3} + 3H_2O$
$Fe(OH)_3$
$HC_2H_3O_2$
$H_2PO_4^-$
$CO_2 + H_2O$
H_2CO_3
HSO_3^-

Fig. 1 - Total Acidity

1) Free Acidity

All acids contain hydrogen; however, certain acid compounds readily dissociate to form H^+ ions in water solution.[1] This dissociated (free) form of the hydrogen ion is known as free acid (Fig. 2) and is a component of industrial wastes and drainage from sulfide-rich terrain.

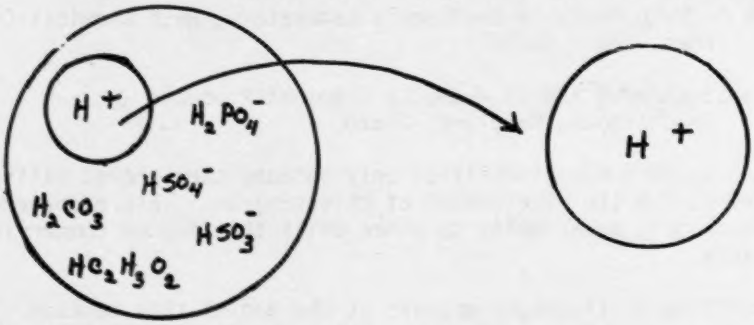

H^+
$H_2PO_4^-$
HSO_4^-
H_2CO_3
HSO_3^-
$HC_2H_3O_2$
H^+

Fig. 2 - Free Acidity vs. Total Acidity

---

[1] The H^+ is bound with water in forms such as H_3O^+ but will be considered as H^+ throughout this guide.

Appendix 1

When hydroxide ions are added to an acidic water sample, they react with the free acid to form water, thus resulting in a decrease in the free acidity (Fig. 3). The quantity of hydroxide ions needed to reach the methyl orange end-point is considered a measure of free acidity.

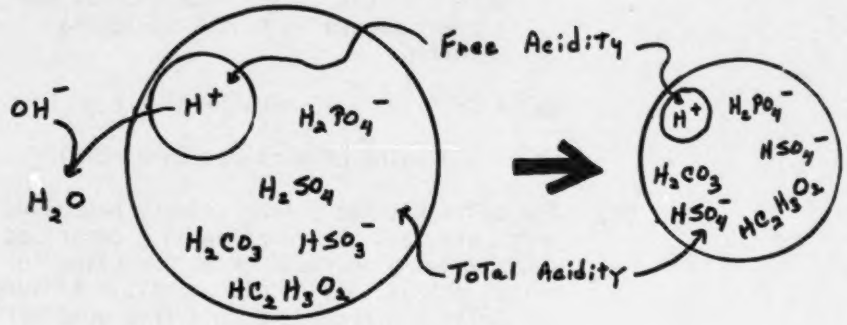

Free Acidity
OH^-
H^+
$H_2PO_4^-$
H_2O
H_2SO_4
H_2CO_3
HSO_3^-
$HC_2H_3O_2$
Total Acidity
H^+
$H_2PO_4^-$
HSO_4^-
H_2CO_3
HSO_4^-
$HC_2H_3O_2$

Fig. 3 - Free Acid Titration

a) Procedure

(1) The Hach and LaMotte kits do not provide instructions for free acidity determinations. However, it is possible to extend their CO_2 procedures to include free acidity measurements by titrating to a methyl orange end-point before going on the phenolphthalein end-point as follows:

1. Prepare the sample as described in Step 1 of the kit procedures.

2. Add 1 drop of methyl orange indicator (see (3) below). If the solution is orange-yellow, the free acidity is not measurable. Continue with step 2 of the kit procedure if a bound acidity value is desired.

3. Titrate with the titration reagent designated for the kit's CO_2 procedure until the orange-yellow end-point is obtained. If chlorine residuals interfere with the end-point determination, add 1 drop 0.1M sodium thiosulfate to a new sample and repeat stages 2 and 3.

4. Record the volume of titrant used and calculate the free acidity in the same manner as described for the CO_2 procedure. Both kits use sodium hydroxide as the titrant according to the following reaction:

$$Na^+ + OH^- + H^+ + X^- = Na^+ + X^- + H_2O$$

(X^- = any anion of a titrateable acid)

(2) The Delta kit has a free acidity procedure which utilizes the reaction just described but substitutes bromcresol green indicator for methyl orange. It is also possible to adapt the Delta CO_2 procedure to a free acidity determination as described in (1).

(3) An alternate procedure to (1) is available as follows:

Equipment:

25 ml graduated cylinder

medicine droppers

50 ml Erlenmeyer flask

burette or 1-ml pipette graduated in 0.1 ml units

Reagents:

Methyl Orange Indicator: Dissolve 0.5 g methyl orange in 1 liter of distilled water.

0.1M Sodium Thiosulfate: Dissolve 2.5 g $Na_2S_2O_3 \cdot 5\,H_2O$ in 100 ml of distilled water.

0.02M NaOH: Prepare 1M NaOH by dissolving 4 g NaOH in 100 ml of FRESHLY BOILED distilled water. Then dilute 2 ml of the stock solution to a 100 ml volume with FRESHLY BOILED distilled water.

Method:

1. Measure 10 ml of sample into the 50 ml Erlenmeyer flask.
2. Add 1 drop of methyl orange indicator. If the solution is orange-yellow, the free acidity is not measurable and should be reported accordingly.
3. Titrate the sample with .02M NaOH. Record the ml needed to reach the orange end-point. If chlorine residuals interfere with the end-point determination, add 1 drop 0.1M sodium thiosulfate to a new sample and repeat stages 2 and 3. (A reference for the end-point can be prepared by adding 1 drop of methyl orange to 10 ml of pH 4.5 solution prepared by combining 1.36 g $NaC_2H_3O_2 \cdot 3H_2O$, sodium acetate, and 10 ml 1M $HC_2H_3O_2$ with distilled water to make 100 ml solution.)

Calculations:

For uniformity, acidity is expressed as $CaCO_3$ equivalents, even though no $CaCO_3$ may be present. The equation for calculating free acidity is

$$\text{mg } CaCO_3/l = \frac{(A)\ (\text{Molarity of NaOH})}{\text{sample volume}} \cdot 50{,}000$$

If: Molarity of NaOH = 0.02M

Sample Volume = 10 ml

A = ml of 0.02M NaOH needed to attain the methyl orange end-point,

Then: mg $CaCO_3/l$ = A x 100

2) Un-ionized (Bound) Acidity (CO_2 Determination)

The acids in the larger circle of Fig. 2 account for un-ionized acidity. However, CO_2 is primary contributor to un-ionized acid levels in most samples ($H_2O + CO_2 = H_2CO_3$). Carbon dioxide commonly enters water via absorption from the atmosphere and as an end-product of both aerobic and anaerobid biological oxidation and respiration.

Once the free acidity is decreased sufficiently by reaction with hydroxide ions, weak acids such as carbonic acid begin to release their hydrogen as free hydrogen ions (Fig. 4).

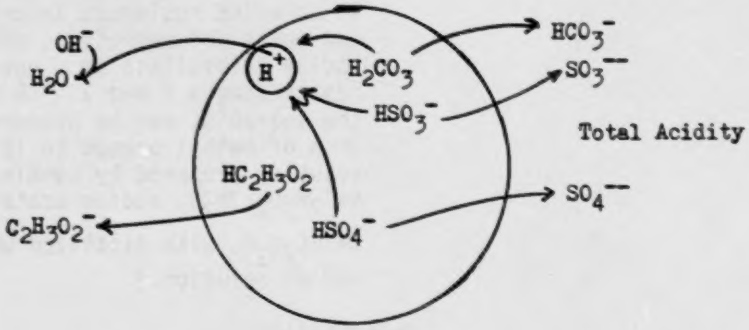


Fig. 4 - Weak Acid Titration

When enough hydroxide ions are added to reach the phenolphthalein end-point, these substances will yield most of their bound hydrogen. Consequently, a quantitative evaluation of un-ionized acidity is achieved by calculating the amount of hydroxide added.

a) Procedure

(1) The Hach, LaMotte, and Delta procedures are actually evaluations of total acidity (free and un-ionized). The free acidity in water which has a pH greater than 4.5 is not measurable. However, if the pH is less than 4.5, free acidity determinations must be completed and then subtracted from the total value obtained by means of this procedure. The reactions are:

$$2\ NaOH + H_2CO_3 = 2H_2O + Na_2CO_3 \qquad (1)$$

$$H_2O + Na_2CO_3 + CO_2 = 2\ NaHCO_3 \qquad (2)$$

Reactions (1) and (2) are employed if NaOH is the titrant. Only reaction (2) is employed if Na_2CO_3 is the titrant.

(2) The following procedure is suggested as an alternate.

Equipment:

See (3) under Free Acidity.

Reagents:

Phenolphthalein Indicator: Place 0.5 g phenolphthalein in 50 ml denatured ethanol and dilute to 100 ml with distilled water.

0.1M Sodium Thiosulfate: Refer to Free Acidity, part (3) for preparation.

0.02M NaOH: Refer to Free Acidity, part (3).

Method:

1. Measure 10 ml of the sample into the 50 ml Erlenmeyer flask.
2. Add one drop of phenolphthalein indicator. (If the solution turns pink, there is no measurable acidity.) Titrate with 0.02M NaOH until the pink phenolphthalein end-point is reached.

Calculations:

Acidity is expressed as mg/l $CaCO_3$. The un-ionized fraction may be calculated according to the following equation:

$$\text{mg } CaCO_3/l = \frac{(B)\ (\text{Molarity of NaOH})}{\text{sample volume}} \cdot 50{,}000$$

If: Molarity of NaOH = 0.02M

Sample Volume = 10 ml

B = ml of 0.02M NaOH needed to attain the phenolphthalein end-point after completing the methyl orange titration

Then: mg $CaCO_3/l = B \times 100$

3) Total Acidity

Total acidity includes all hydrogen ion donors measured by titration of a water sample to the phenolphthalein end-point.

a) Procedure

(1) The CO_2 procedures in the Delta, Hach and LaMotte kits will give total acidity evaluations without modification.

(2) An alternate approach is to combine the alternate procedures suggested for free un-ionized acidity as follows:

1. Titrate to the methyl orange end-point and calculate free acidity if desired (see (1) on page A-6)

2. Add phenolphthalein and titrate to the phenolphthalein end-point (see (2) on page A-7)
Calculate the total acidity as mg $CaCO_3/l = C \times 100$, where C = total volume of titrant used.

4) Reference

Standard Methods for the Examination of Water and Wastewater, (12th ed.), American Public Health Association, New York, 1965, pp. 46-47.

b. Alkalinity

Alkalinity is an indicator of the ability of a given water sample to neutralize or accept hydrogen (H^+) ions. Some of the substances which comprise or contribute to alkalinity within the pH range of 4.5 to 11 are depicted in Fig. 1.

The circle on the left of Fig. 1 includes several substances which accept hydrogen ions directly during alkalinity measurements (titrateable alkalinity). The circle on the right includes substance which undergo chemical changes such as the hydrolysis of water which produce hydrogen ion acceptors. Those chemical species within the overlap of the two circles may serve in both capacities. Hydroxide, carbonate, and bicarbonate ions are normally the predominating members of their respective groups.

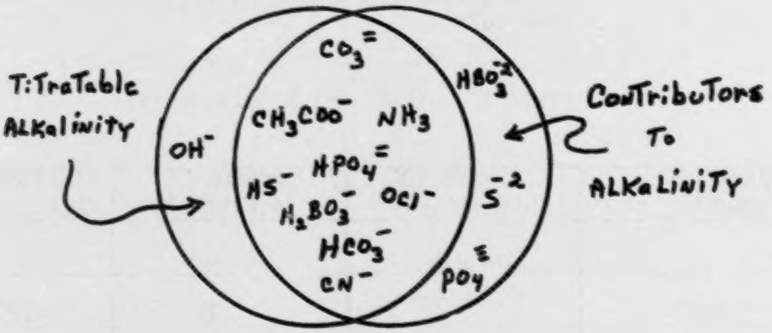


Fig. 1 - Components of Alkalinity
(pH 4.5 to 11)

Alkalinity is determined by titrating samples which are alkaline to phenolphthalein to the phenolphthalein end-point with sulfuric acid. This serves as a measure of the "phenolphthalein alkalinity" which includes nearly all hydroxides and half of the carbonates present. Titration is then continued beyond the phenolphthalein end-point to the methyl orange or bromcresol green-methyl orange. This step of the titration neutralizes the remaining half of the carbonates and the bicarbonates. The addition of the sulfuric acid volume needed to reach the phenolphthalein end-point to the amount needed to reach the methyl orange end-point leads to a calculation of the "total alkalinity."

Appendix 1

Sometimes it is desirable to attempt a calculation of the concentrations of individual contributors to alkalinity. Simplified calculation procedures summarized in Table 1 are based upon the following concepts:

(1) Hydroxides, carbonates, and bicarbonates are usually the major sources of alkalinity in natural waters.

(2) Hydroxides and bicarbonates are incapable of existing together in the same solution. (Assumed, but not true.)

(3) The hydroxide supply is essentially exhausted by titration to the phenolphthalein end-point.

(4) One-half of the carbonates is titrated upon reaching the phenolphthalein end-point.

(5) The bicarbonates and the remaining half of the carbonates are titrated when proceeding from the phenolphthalein end-point to the methyl orange end point.

Table 1. Alkalinity Relationships

TITRATION RESULT	HYDROXIDE ALKALINITY	CARBONATE ALKALINITY	BICARBONATE ALKALINITY
P=T	equals T	0	0
P < 1/2T	0	2P	T-2P
P=1/2T	0	T	0
P > 1/2T	T -2 (T-P) or 2P-T	2(T-P)	0
P=0	0	0	T

P = Phenolphthalein Alkalinity T = Total Alkalinity

1) Procedure

a) Refer to Delta, Hach, and LaMotte kits. The reactions are classical acid-base neutralizations.

$H^+ + X^- = HX$ (X^- = any anion of a weak acid)

b) The following procedure using available laboratory materials is suggested (1).

Equipment:

25 ml graduated cylinder

medicine droppers

50 ml Erlenmeyer flask

burette or 1 ml pipette graduated in 0.1 ml units

Reagents:

Methyl Orange Indicator: Dissolve 0.5 g of methyl orange in 1 liter of distilled water.

Phenolphthalein Indicator: Place 0.5 g of phenolphthalein into 50 ml of denatured ethanol and dilute to 100 ml.

0.1M Sodium Thiosulfate: Dissolve 2.5 g of $Na_2S_2O_3 \cdot 5H_2O$ in 100 ml of distilled water.

0.01M Sulfuric Acid: Add 3 ml of concentrated H_2SO_4 (18M) to 1 liter of distilled water, yielding 0.05M H_2SO_4. Dilute 20 ml 0.05M H_2SO_4 to 100 ml yielding 0.01M H_2SO_4.

Method:

1. If present, remove free residual chlorine by adding 1 drop of sodium thiosulfate to a 100 ml sample.
2. Measure a 10 ml sample into the titration flask and add 1 drop of phenolphthalein. If solution is not pink, no free alkalinity is present. Skip step 3 and proceed to step 4.
3. Add 0.01M sulfuric acid to the sample with the pipette or burette. Record the number of mls needed to reach the pink end-point. Use this number in the calculation of phenolphthalein alkalinity.
4. Add 1 drop of methyl orange indicator.

5. Continue to titrate with 0.01M sulfuric acid until the methyl orange end-point is reached. Record the volume (ml) used and combine this value with the volume (ml) obtained in step 3. Use this value for the calculation of total alkalinity. (A reference for the end-point can be prepared by adding 1 drop of methyl orange to 10 ml of pH 4.5 solution prepared by combining 1.36 g $NaC_2H_3O_2 \cdot H_2O$ and 10 ml 1M $HC_2H_3O_2$ with water to make 100 ml solution.)

Calculations:

For uniformity, alkalinity is expressed as mg $CaCO_3$/l even though there may be no $CaCO_3$ present. The equation for the phenolphthalein alkalinity is

$$\text{mg } CaCO_3/l = \frac{A \times (\text{Molarity of } H_2SO_4) \times 100{,}000}{\text{Volume of Sample}}$$

where A equals ml of the titrant used to reach the phenolphthalein end-point and the concentration of the sulfuric acid is expressed as molarity.

This can be reduced to:

$$\text{mg } CaCO_3/l = A \times 100$$

if a 10 ml sample is used and the sulfuric acid is 0.01M.

In the same way, the total alkalinity is calculated as

$$\text{mg } CaCO_3/l = B \times 100$$

where B is the TOTAL number of ml needed to reach the methyl orange end-point.

2) Reference

Standard Methods for the Examination of Water and Wastewater, (12th ed.), American Public Health Association, New York, 1965, pp. 48-50.

c. pH

pH is a measurement which reflects the instantaneous free hydrogen ion concentration in a water sample. Free hydrogen and hydroxide ions exist in equilibrium in all aqueous solutions. If these ions are present in equal amounts, the sample is described as neutral and has a pH value of 7. If the hydrogen ion concentration is less than the hydroxide ion concentration, the solution is said to be basic and has a pH value greater than 7. If the hydrogen ion concentration is greater than the hydroxide ion concentration, the solution is acidic and has a pH value less than 7 (Fig. 1).

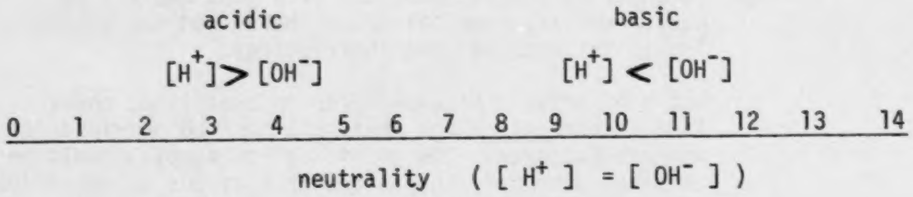


Fig. 1 - pH Relationships

It is essential to regognize that pH is not a measurement sensitive to the presence of substances which may contribute to the total acidity or alkalinity of a given sample. Consequently, it must not be confused with the results of total acidity and alkalinity determinations. Samples which possess a neutral pH may possess high acidity and/or alkalinity values. Because natural waters are buffered by the CO_2, HCO_3, CO_3 system to a pH range of 6.5 to 7.5, marked deviations from neutrality are generally the result of industrial or acid mine contamination.

The pH of water samples is usually determined by either colorimetric or electrometric techniques. Colorimetric procedures rely upon chemical substances which undergo color changes with change in pH. There are numerous reagents which demonstrate this phenomenon; however, each is effective as a pH indicator within a limited pH range only. A versatile pH measurement system must contain numerous indicators covering the entire pH spectrum. These indicators are either impregnated on paper strips, used separately in solution form, or combined to create a "universal" or "wide-range" indicator solution.

Electrometric techniques yield the greatest accuracy. They employ meters which, by means of a glass electrode, detect differences in electric potential which occur with differing pH values. Once the meter is properly calibrated, pH readings are read directly from the instrument scale.

Procedure:

1) Refer to the Delta, Hach or LaMotte kits. All three utilize colorimetric procedures.

2) As alternatives to the kits, the following procedures are recommended:

   a) Purchase universal indicator or a good quality pH paper from any chemical supply house and use according to the accompanying instructions.

   b) Use a pH meter. Models differ in operation; therefore, instructions for their use must be obtained from the manufacturer. The pH of a given sample should be obtained propmptly to prevent changes due to reactions with CO_2 from the air or loss of CO_2 to the air.

2. Dissolved Gases

   a. CO_2 - See end of this reference section for material.

   b. Chlorine (residual)

Both free and combined forms of chlorine are used as disinfectants in attempts to curb waterborne diseases. Chlorine does not occur naturally in water but may enter through sewage treatment effluents and industrial wastes.

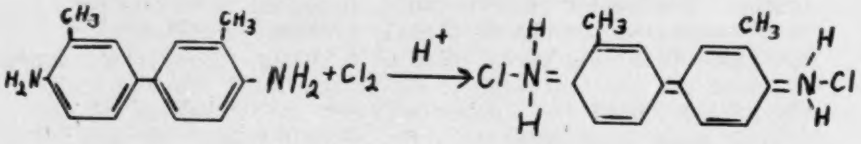
In the quantitative determination of chlorine, an organic compound orthotolidine is oxidized in acid solution by both free and combined forms of chlorine. This produces a yellow colored compound holoquinone, which is measured colorimetrically.

An alternate method which corrects for color interferences is known as the orthotolidine-arsenite method. Total residual chlorine is measured in the usual way with orthotolidine as described above. A second test which serves as a blank is prepared by introducing sodium arsenite solution before adding the orthotolidine. The arsenite, being a much stronger reducing agent than orthotolidine, reduces both free and combined chlorine. This prevents their reaction with the orthotolidine. Any color present in this second test is due to interference by other chemical substances and the reagents being used. The total residual chlorine level can be calculated as follows:

Total chlorine residual (Test 1)	and	Interfering Color (Test 2)	=	Total Residual Chlorine

1) Procedure

a) Refer to Delta kit. For clear waters, Delta uses the orthotolidine method. The reaction is as follows:



orthotolidine

A holoquinone (yellow)

For turbid waters, Delta uses the orthotolidine-arsenite method as described above.

b) Refer to Hach kit. Because of color fading, Hach has developed a modified orthotolidine reagent called O-ToliVer which stabilizes the final color for longer periods of time. The reaction is similar to that in the Delta kit.

c) Refer to the LaMotte kit. It uses the orthotolidine method as described above.

c. Dissolved Oxygen

Dissolved oxygen is an essential substance for the support of most aquatic life. Its concentration in water (normally very low compared with that in air) varies with fluctuations in such factors as temperature, types and concentrations of dissolved and suspended solids, biotic activity, and agitation of the water. Both depressed and elevated (supersaturated) dissolved oxygen levels are encountered in aquatic studies. In view of our understanding of the biological role of DO, deleterious effects of low or nonexistent levels of DO are hardly surprising. Harmful effects accompanying DO supersaturation of water supplies have not been so readily anticipated. However, fish have demonstrated low tolerance to DO supersaturation as indicated by an increased incidence of mortality and disease in such waters (1,2).

Regardless of the test used for determination of DO, the sampling procedures must avoid aeration and warming. Moreover, the test must be done immediately or the oxygen must be fixed if chemical and biochemical influences are to be avoided. The Azide-Winkler method, an accurate and feasible test for DO, eliminates interference by nitrite ions through the use of sodium azide. Dissolved oxygen is fixed by the addition of manganese sulfate and an alkali-iodide-azide reagent. In this reaction, the oxygen oxidizes manganous ions to manganese oxyhydroxide; $MnO(OH)_2$. Under acid conditions (obtained by adding concentrated sulfuric acid or the less dangerous solid form of sulfamic acid), the manganese oxyhydroxide oxidizes iodide ions to produce free iodine. The amount of free iodine produced is equivalent to the dissolved oxygen originally present. Following titration to a pale straw color with sodium thiosulfate, starch is added and the titration is continued until the blue color disappears. With clean water samples, the titration may be delayed under acid conditions for up to 6 hours. Prompt titration is required for polluted water.

1) Procedure

a) Azide-Winkler method (in lab without kit)[3]

(1) Equipment

4-5 ml pipettes

burette, in 0.1 ml units with a 50 ml capacity

BOD bottles, 300 ml capacity

Erlenmeyer flask, 250 ml

(2) Reagents

Manganese Sulfate Solution: Dissolve 480 g $MnSO_4 \cdot 4H_2O$ in distilled water, filter and dilute to 1 liter.

Alkali-iodide-azide Reagent: Dissolve 500 g NaOH and 150 g KI in distilled water and dilute to 1 liter. To this solution add 10 g NaN_3 dissolved in 40 ml of distilled water. This reagent should not give a color with starch solution when diluted and acidified.

Concentrated Sulfuric Acid: Use concentrated reagent grade acid (H_2SO_4). Handle carefully!

Starch Solution: Prepare a paste of 5-6 g soluble starch in a small amount of distilled water. Pour this paste into 1 liter of boiling distilled water, allow to boil a few minutes and let settle over night. Use the clear supernate.

Sodium Thiosulfate Solution: Dissolve 24.82 g $Na_2S_2O_3 \cdot 5H_2O$ in boiled and cooled distilled water and dilute to 1 liter. Preserve by adding 0.4 g of NaOH per liter.

Working Sodium Thiosulfate Titrant 0.0375M: Prepare by either diluting 375 ml sodium thiosulfate stock solution to 1 liter or by dissolving 9.30 g $Na_2S_2O_3 \cdot 5H_2O$ in freshly boiled and cooled distilled water and dilute to 1 liter. (For standardizing the sodium thiosulfate, refer to Standard Methods, p. 407.)

b) In the field

(1) Fill a 300 ml glass stoppered bottle with sample water by allowing the sample to enter through a glass or rubber tube which extends to the bottom of the bottle. An overflow displacing the bottle contents 2-3 times is necessary to ensure that the test sample has not been exposed to the air. Stopper the bottle immediately upon removing the tube. Be sure that no bubbles are trapped within the bottle.

(2) Add 2 ml manganese sulfate to the collecting bottle by means of a pipette inserted just below the surface of the liquid.

(3) Add 2 ml alkali-iodide-azide reagent in the same manner.

(4) Stopper with care to exclude air bubbles and mix by inverting the bottle several times. When the precipitate settles shake again and allow to settle.

Note: The oxygen is fixed according to the following reaction.

$$Mn^{++} + 2\,OH^- + 1/2\,O_2 \longrightarrow \underset{\text{(golden brown flocculant)}}{MnO(OH)_2} \quad \text{(eq. 1)}$$

c) In the lab

(1) Add 2.0 ml concentrated H_2SO_4 with the pipette above the surface of the liquid; stopper and invert several times to dissolve the precipitate.

Note: With the addition of sulfuric acid, the proper low pH conditions are obtained for the destruction of interfering NO_2^- by the sodium azide which was added in the alkali-iodide-azide reagent above. The following reactions occur:

$$NaN_3 + H^+ \longrightarrow HN_3 + Na^+ \qquad \text{(eq. 2)}$$

$$HN_3 + NO_2^- + H^+ \longrightarrow N_2 + NO_2 + H_2O \qquad \text{(eq. 3)}$$

Under the same pH conditions, the Mn^{+4} oxidizes I^- to produce free I_2 as follows:

$$Mn\,O(OH)_2 + 2\,I^- + 4\,H^+ \longrightarrow Mn^{++} + I_2 + 3H_2O \qquad \text{(eq. 4)}$$

(2) In an Erlenmeyer flask, titrate the 300 ml sample with 0.0375M sodium thiosulfate to a pale straw color.

(3) Add 2 ml of starch solution. A blue color forms indicating the presence of molecular iodine, I_2. Continue titrating until the molecular iodine is reduced to iodide ions as indicated by the disappearance of the blue color.

Note: The reaction is

$$2\,S_2O_3^{--} + I_2 \longrightarrow S_4O_6^{--} + 2\,I^- \qquad \text{(eq. 5)}$$

(4) Record the total amount of sodium thiosulfate used.

2) Calculations

1 ml of 0.0375M $Na_2S_2O_3$ is equivalent to 0.2 mg DO per 300 ml sample as follows:

According to (eq. 5), $S_2O_3^{--}$ loses 1 electron so that 1 liter of 0.0375M $Na_2S_2O_3$ will lose 0.0375 moles of electrons (or 1 ml will lose 3.75×10^{-5} moles of electrons). To change 1 mole of molecular oxygen (O_2) to O^{--} requires 4 moles of electrons. 3.75×10^{-5} moles of electrons will reduce approximately 9.4×10^{-6} moles of molecular O_2.

Since 1 mole of O_2 has a mass of 32 g, 9.4×10^{-6} moles has a mass of 0.0003 g or 0.3 mg. Each milliliter of sodium thiosulfate used in the titration of a 300 ml sample indicates the presence of 0.3 mg O_2/300 ml or 1 mg O_2/liter (1 ppm).

Summarizing: Each ml of sodium thiosulfate added in steps 3 and 4 equals 1 mg/l DO (1 ppm).

3) As an alternative to the laboratory method described above, refer to either the Hach or LaMotte kits. They utilize chemical principles outlined for the laboratory method with exceptions as follows:

   a) Hach: Substitutes phenylarsene oxide (PAO) for the sodium thiosulfate titrant.

   b) LaMotte: Utilizes an unmodified Winkler procedure; consequently, it is subject to interference by nitrite ions.

4) References

   (1) McKee, J. E. and H. W. Wolf, Water Quality Criteria, (2nd ed.), State Water Quality Control Board, Pub. #3-A, Sacramento, Calif., 1963, p. 181.

   (2) *Ibid*.

   (3) Standard Methods for the Examination of Water and Wastewater, (12th ed.), American Public Health Association, New York City, 1965, pp. 415-419.

   (4) Carbon Dioxide prior to Chlorine
   Refer to Section 2), Un-ionized (Bound) Acidity, Acid-Base Parameters (see page A-5).

3. Dissolved and Suspended Solids

a. Chloride

The chloride ion is a component of many salts and most living organisms. Because chloride salts are usually soluble, ions find their way into natural waters by phenomena such as erosion and leaching. Examples of other common chloride sources include sea water intrusion, human and animal sewage, fertilizers, industrial wastes, and winter salting of highways.

Gradually add mercuric nitrate or silver nitrate solution to a water sample containing an indicator. The mercuric or silver ions combine with the chloride ions until the chloride supply is essentially depleted. At this point, mercuric or silver ions form a colored complex by reacting with the indicator. The amount of mercuric nitrate or silver nitrate solution added indicates the chloride ion concentration.

1) Procedure

a) Refer to the Hach, Delta, or LaMotte kits if they are available. These kits utilize the following reactions:

Hach Kit: (1) $Hg^{++} + 2Cl^{-} = HgCl_2$

(2) Hg^{++} + Diphenylcarbazone = purple complex

Delta & LaMotte Kits:

(1) $Ag^{+} + Cl^{-} = AgCl$

(2) $2\ Ag^{+} + CrO_4 = Ag_2CrO_4$ (red color)

b) If a commercial kit is not available, the following procedure which uses the above reactions is suggested.

Equipment:

burette, 25 ml

porcelain evaporating dish, 250 ml

glass stirring rod

assorted beakers, graduates, one-liter volumetric flasks, and bottles as needed

five ml pipette

Reagents:

Silver Nitrate, 0.0141M: Dissolve 2.396 g silver nitrate ($AgNO_3$) in distilled water and dilute to 1 liter in a volumetric flask. Standardize against 0.0141M sodium chloride solution. One ml silver nitrate solution equals approximately 0.500 ml Cl^{-}.

Sodium Chloride, 0.0141M: Dissolve 0.8241 g of sodium chloride (NaCl) in distilled water and dilute to 1 liter in a volumetric flask. One ml sodium chloride solution equals 0.500 mg Cl^{-}.

Potassium Chromate Indicator: Dissolve 50 g potassium chromate (K_2CrO_4) and dilute to 1 liter with distilled water.

Method:

1. To standardize the silver nitrate, add 20 ml of 0.0141M sodium chloride to 1 ml of potassium chromate indicator in a porcelain evaporating dish. Titrate as per step 4 below. Then calculate the normality constant as follows:

   $$\frac{\text{ml } AgNO_3}{20} \times 500 = \text{Normality constant}$$

2. Place 100 ml sample or a smaller quantity diluted to 100 ml with distilled water in a porcelain evaporating dish.
3. Add one ml of potassium chromate indicator with a pipette.
4. Add silver nitrate solution from a burette, stirring the dish contents until a uniform pinkish-yellow end-point is reached. Record the ml of silver nitrate added.
5. Repeat steps 2, 3, and 4 above using 100 ml of distilled water as a blank in place of the sample.
6. Calculate the final result as follows:

$$\text{mg/l } Cl^- = \frac{(\text{ml } AgNO_3 \text{ for sample} - \text{ml } AgNO_3 \text{ for blank})\ (\text{Normality Constant})}{\text{ml original sample}}$$

2) Reference

Water Pollution Control Federation, Simplified Laboratory Procedures for Wastewater Examination, WPCF Publication, No. 18, 1968, pp. 45-46.

b. Hardness--Calcium, Magnesium, Total

Hardness is a water quality parameter which limits the lathering or foaming ability of soaps and increases the tendency of a water sample to produce scale in pipes, heaters, and boilers. Hard water is caused by the presence of divalent ions such as

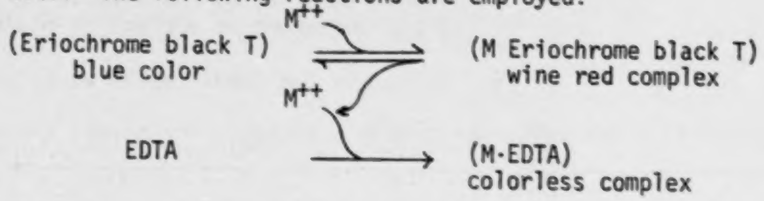
Calcium (Ca^{++}) and magnesium (Mg^{++}). Additional ions (e.g., Sr^{++}, Mn^{++}, Fe^{++}) can cause hardness but are present only in limited amounts in most water supplies. If their concentrations are elevated, they should be included in calculations of total hardness. All of these cations enter water sources via industrial wastes, sewage, and contact with soil and rock formations.

The chemical determination of total hardness involves the titration of a water sample to which an indicator, such as Eriochrome black T, has been added. The substance EDTA is used as the titrant because of its ability to complex with divalent cations. Prior to titration, the indicator forms a red complex with Ca^{++} or Mg^{++}. During titration within a specific pH range, the red indicator releases its bound cations to the EDTA and reverts to its blue pigment. Total hardness is calculated from the amount of EDTA needed to reach the blue end-point.

In the determination of calcium hardness, magnesium is precipitated as magnesium hydroxide by the addition of alkali. The rest of the procedure is completed as outlined above. Magnesium hardness is calculated by subtracting the calcium value from the total hardness figure.

1) Procedure

   a) For total hardness, refer to the Hach or LaMotte kits. The following reactions are employed:



   M^{++} = any divalent cation

   EDTA = ethylenediamine tetraacetic acid

   b) For calcium hardness, refer to the Hach, LaMotte or Delta kits. Following the addition of sodium hydroxide or potassium hydroxide to precipitate magnesium hydroxide, the following reaction occurs:

$$Mg^{++} + 2\ OH^{-} = Mg\ (OH)_2$$

   The reactions obtained from the Hach and LaMotte kits were described above. While the Delta kit uses different reagents, it appears to utilize a similar process.

c) Magnesium hardness may be calculated by determining the difference between the total hardness and calcium hardness value.

d) As an alternate procedure, calcium hardness may be evaluated in a rough quantitative fashion by the following precipitation procedure according to the reaction:

$$Ca^{++} + C_2O_4^{--} = CaC_2O_4 \underset{\text{(solid)}}{(s)}$$

Equipment:

2 test tubes

4 dropping pipettes

Reagents:

Stock 0.01M Ca^{++} Solution: Add 1.11 g of $CaCl_2$ to 100 ml of distilled water and dilute to 1 liter.

Working Ca^{++} Standard (80 ppm Ca^{++}): Add 20 ml of the stock solution to 80 ml of distilled water.

Concentrated Ammonia Water.

4% Ammonium Oxalate: Dissolve 4 g of $(NH_4)_2C_2O_4$ in 50 ml of distilled water and dilute to 100 ml.

Method:

1. Prepare a reference sample containing Ca^{++} by placing 20 drops (1 ml) of the working Ca^{++} standard into Tube 1.
2. Place 20 drops of the water sample into Tube 2.
3. Add two drops of concentrated ammonia water to both tubes.
4. Add 4% ammonium oxalate dropwise until a reaction is observed. Do not add more than 5 drops.
5. Compare the amount of precipitation in Tube 2 with that in Tube 1. Report your result as being greater than or less than 80 ppm.

2) Reference

Standard Methods for the Examination of Water and Wastewater, (12th ed.), American Public Health Association, New York City, 1965, p. 149.

c. Iron

Ionic forms of iron occur in water as either the iron (II) or iron (III) form. Iron (II) is easily oxidized to iron (III) which reacts with hydroxides to form insoluble iron (III) hydroxide, thus keeping iron concentrations in most water supplies at low levels. While toxic to many organisms, elevated iron concentrations support iron bacteria (which may cause corrosion) in pipe lines or structures with formation of slimes, pits, encrustations, and other undesirable effects. Dissolved iron originates from soils or rock formations during leaching and erosion processes effected by acidic water flows. Also, there is evidence which suggests that iron enters water sources through changes produced in environmental conditions as a result of biological reactions.

In quantitative iron studies, it is necessary to convert all of the iron (III) to the soluble iron (II) form. This is accomplished by dissolving any precipitated iron (III) hydroxide by the addition of hydrochloric acid and reducing the iron (III) species to iron (II) through the action of hydroxylamine, a strong reducing agent. The water sample is then treated with 1,10-phenanthroline which combines with the iron (II) to form an orange-red complex suitable for colorimetric evaluation.

An alternative procedure involves the conversion to iron (II), as described, followed by the addition of ethylenediamine which buffers the water sample and complexes* heavy metals which might give erroneously high results. 2,2,2-tripyridine is added to yield a reddish-purple iron (II) complex for colorimetric study.

1) Procedure

Refer to the Hach, Delta, or LaMotte kits. The following reaction sequences are used:

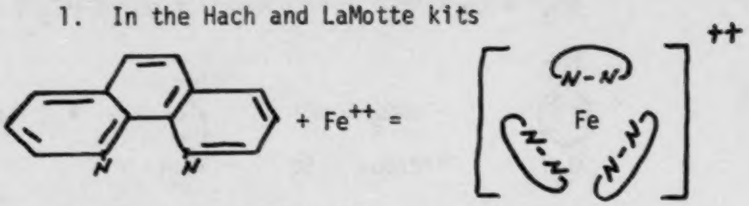
$$Fe(OH)_3 + 3\ H^+ \longrightarrow 3H_2O + Fe^{+++}$$

$$4\ Fe^{3+} + 2\ NH_2OH = 4\ Fe^{++} + N_2O + H_2O + 4\ H^+$$

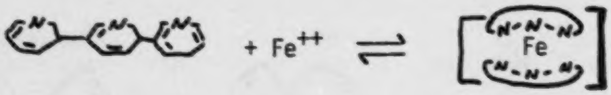
* binds up

These are followed by:

1. In the Hach and LaMotte kits



2. In the LaMotte kit:



2) Reference

Sawyer, C. N.,and P. L. McCarty, Chemistry for Sanitary Engineers, (2nd ed.), McGraw-Hill Book Co., New York City, 1968, pp. 446-448.

d. Nitrate

Nitrate ions are end-products of the oxidation of nitrogen or nitrogen compounds. They are formed by (1) the nitrogen fixation activity of certain bacteria and algae, (2) the oxidation of atmospheric nitrogen during electrical storms, and (3) the oxidation of nitrogenous compounds (ammonia, nitrates, proteins, certain organics) in both water sources and aerobic sewage treatment systems. Their use in fertilizers as a source of nitrogen for plant protein synthesis constitutes a source of pollution, as excess amounts are carried into water supplies by percolation and runoff.

In the suggested procedures, nitrates are measured by reduction by cadmium to nitrite ions followed by reaction with sulfanilic acid to form a diazonium salt. The salt is reacted with 1-naphylamine hydrochloride to form a red-colored azo dye.

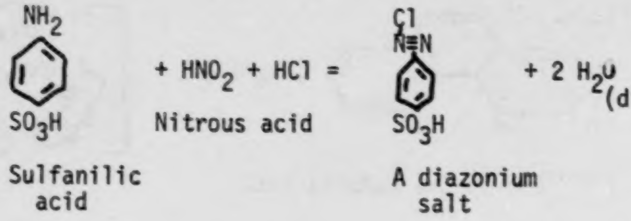
The presence of nitrite ions in the original water sample will cause falsely high nitrate values. A correction is achieved by measuring the nitrite level separately (see Nitrite) and subtracting the resulting nitrite value from the nitrate value obtained in the cadmium reduction method just described.

1) Procedure

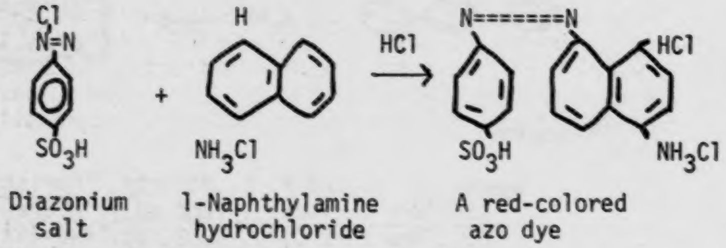
Refer to the Hach, Delta, or LaMotte kits.

Their reactions are:

$NO_3^- + Cd = NO_2^- + CdO$ (reduction of NO_3^-) (Eq. 1)


NH2
SO3H
Sulfanilic acid
+ HNO2 + HCl =
Nitrous acid
Cl
N≡N
SO3H
A diazonium salt
+ 2 H2O

(Eq. 2) (diazotization)


Cl
N=N
SO3H
Diazonium salt
+
H
NH3Cl
1-Naphthylamine hydrochloride
HCl
N=======N
HCl
SO3H
NH3Cl
A red-colored azo dye

(Eq. 3)

e. Nitrite

Nitrites are intermediates in the chemical or biological modification of nitrogenous compounds such as ammonia, nitrates, certain organics, dyes, and proteins. Accordingly, they may occur in water supplies containing such substances.

Nitrites are measured by conversion to a diazonium salt through reaction with sulfanilic acid. Upon reaction with 1-naphthylamine hydrochloride, a red-colored dye develops which is easily measured by colorimetric procedures.

Procedure

Refer to the Hach, Delta, or LaMotte kits. The chemistry is described by Equations (2) and (3) of the Nitrate Ion section.

f. Phosphate

The phosphate ion exists in both organic and inorganic forms. With the exception of bottom sediments, and samples containing algae and suspended particles which may possess organic phosphorous as a major phosphorous form, emphasis is placed on analystical evaluations of the inorganic forms outlined in Table 1.

Table 1. - Inorganic Phosphates

Polyphosphates*	Orthophosphates*
(meta) $(MPO_3)x$	MH_2PO_4
(pyro) $M_4P_2O_7$	M_2HPO_4
(tri) $M_5P_3O_{10}$	M_3PO_4
(tetra) $M_6P_4O_{13}$	

* M = any monovalent cation

These determinations are considered significant because of our increased awareness of the role of phosphates in life processes (ATP, enzyme function, buffering) combined with their extensive use in fertilizers, detergents, water softeners and as nutrients in the biological degradation of sewage.

The suggested procedures detect only orthophosphates; consequently, it is necessary to convert the polyphosphates to the ortho form if a reliable measure of the inorganic phosphates content is to be obtained. This process occurs in all aqueous systems but may take from hours to several days for completion under field conditions. In the laboratory, the conversion is hastened by boiling the sample in an acidic solution. If organic phosphorous is to be included in the analysis, it must be converted to the orthophosphate form through oxidation by sodium persulfate (refer to Standard Methods).

Detection of the orthophosphate form is accomplished by reacting it with ammonium molybdate to form ammonium phosphomolybdate. This product is subsequently reduced to molybdenum blue by reaction with stannous ions.

1) Primary Procedure

Refer to the Hach, Delta, or LaMotte kits if they are available.

a) $PO_4^{-3} + 12\,(NH_4)_2\,MoO_4 + 24\,H^+ = (NH_4)_3PO_4\cdot 12MoO_3 + 21\,NH_4^+ + 12\,H_2O$

b) $(NH_4)_3PO_4\cdot 12MoO_3 + Sn^{++} = \text{(molybdenum blue)} + Sn^{+4}$

2) The following alternative procedure for orthophosphate only is suggested. The reactions just described are utilized.

Equipment:

2 test tubes

3 medicine droppers

Reagents:

Stock 0.001M Phosphate Solution: Add 0.136 g KH_2PO_4 to distilled water making total volume 1 liter.

Working Standard ppm H_2PO_4: Add 10 ml of stock solution to 990 ml of distilled water.

Ammonium Molybdate - Nitric Acid Reagent: Dissolve 15 g of ammonium molybdate in 300 ml of distilled water. Add 100 ml of nitric acid 1:1 dilution of concentrated HNO_3 and saturate with ammonium nitrate.

Method:

1. Prepare a reference sample containing phosphate ions by placing 20 drops of working standard in Tube 1.
2. Place 20 drops of the water sample into Tube 2.
3. Add 10 drops of the ammonium molybdate-nitric acid reagent to each tube.
4. Add a few crystals of stannous chloride to both tubes. A blue color should appear if orthophosphate ions are present.
5. Compare the intensity of the blue pigment in Tube 2 with that of Tube 1. Report your results as having less than or greater than 1 ppm orthophosphate.

g. Sulfate

The sulfate ion, a complex of sulfur and oxygen, is capable of serving as an oxygen donor for biochemical oxidations occuring under anerobic conditons. This action results in the conversion of the sulfate ion to the sulfide form which equilibrates with hydrogen ions to form hydrogen sulfide. The latter substance possesses an objectionable "rotten egg" odor and is capable of being oxidized by sulfur bacteria to form sulfuric acid. The sulfate ion is derived from sewage, industrial and agricultural effluents, erosion, and percolation of water through pyrite or sphalerite ore deposits.

Analytical techniques for sulfates are based upon the formation of insoluble barium sulfate by the addition of barium ions. The resulting solid may be collected, dried, and weighed or may be kept in colloidal suspension by the use of a conditioning reagent containing hydrochloric acid, sodium chloride, glycerol, and other organic compounds and then measured by turbidimetric procedures. At least one titrimetric procedure is available which involves the gradual addition of barium chloride to a water sample containing an indicator. The barium ions precipitate with the sulfate ions until the sulfate ion supply is essentially depleted. Excess barium ions then combine with the indicator to produce a color change. The sulfate level is calculated from the amount of barium chloride needed to achieve the end-point.

1) Procedure:

a) Refer to the Hach or Delta kits. They utilize the following reactions:

(1) Hach kit (Turbidimetric Procedure)

$Ba^{++} + SO_4^{--} = BaSO_4$ (solid)

(2) Delta kit (Titrimetric Procedure)

$Ba^{++} + SO_4^{--} = BaSO_4$ (solid)

THEN Ba^{++} + Indicator = orange-red complex

b) An alternative rough quantitative procedure is suggested as follows.

Apparatus:

2 test tubes

4 medicine droppers

Reagents:

Stock 0.01M $MnSO_4$ Solution: Add 1.7 g $MnSO_4 \cdot H_2O$ to 100 ml distilled water and dilute to 1 liter.

Working SO_4^{--} Standard (96 ppm SO_4^{--}): Add 10 ml of stock solution to 90 ml of distilled water.

6M Hydrochloric Acid: Dilute concentrated HCl (12M) to 1/2 its original concentration.

0.1M Barium Chloride: Dissolve 2.08 g $BaCl_2$ in 50 ml of distilled water and dilute to 100 ml.

Method:

1. Prepare a reference sample containing SO_4^{--} by placing 10 drops of the working standard into Tube 1.
2. Place 10 drops of the water sample in Tube 2.
3. Add 2 drops of HCl and 1 drop of $BaCl_2$ to each test tube.
4. Formation of a white precipitate or cloudiness indicates the presence of SO_4^{--}. Compare the amount of cloudiness or precipitation in Tube 2 with that in Tube 1 and report your result as greater or less than 96 ppm SO_4^{--}.

h. Turbidity

Turbidity limits light penetration within a body of water by causing incident light to be scattered or absorbed rather than transmitted appreciable distances through the sample. Turbid water is caused by the presence of suspended organic and inorganic solids derived from erosion, surface drainage systems, and domestic and industrial wastes. It exerts a negative influence on photosynthesis and water temperature by reducing the amount of light reaching subsurface areas and can, by itself, kill fish and other organisms. Increases in turbidity may follow a chain reaction sequence by providing bacteria and other microorganisms contributing to turbidity with an abundant supply of nutrients required for growth and reproduction.

Turbidity is measured by comparing the interference to the passage of incident light in the questioned sample with that in a standard reference. Although the accuracy of photometric or nephelometric techniques is questionable, such procedures are convenient for approximating turbidity and are used in most commercial kits.

Procedure:

1) Refer to Hach or Delta kits.

2) The following procedure which measures the depth of light

penetration can be used to supplement photometric determination of turbidity. The depth of light penetration is affected by turbidity, but also color.

a) Equipment (a home-made Secchi disk costs about $.50)

(1) calibrated rope

(2) tempered plywood aide, 20 cm in diameter, with alternate white and black quadrants

(3) eye-bolt, washers

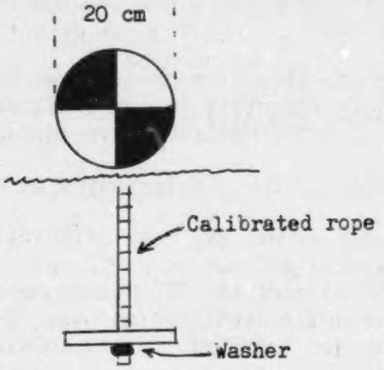


Figure 1.

b) Method

(1) Lower disk and record depth of disappearance.

(2) Lower disk below the recorded point and then slowly raise it. Record the depth at which the disk first becomes visible.

(3) Average the two readings. Secchi disk readings range from a few centimeters to over 40 meters.

4. Oxygen Demand

Biochemical Oxygen Demand (BOD)

BOD values reflect the quantity of molecular oxygen required for the decomposition of organic compounds by aerobic biochemical processes. Consequently, BOD values serve as an index of the pollution strength of wastes by measuring the amount of oxygen which may be removed from water supplies as these wastes are being aerobically stabilized.

Appendix 1

The BOD determination is a bioassay procedure requiring (1) excess O_2, (2) favorable physical conditions, (3) essential nutrients, (4) suitable organisms, and (5) time. While 20 days are usually required to approach complete waste stabilization, the length of the assay is set at 5 days. The shorter period usually allows for the measurement of a substantial fraction of the total BOD. It also minimizes interference by autotrophs, particularly nitrifying bacteria, which aerobically metabolize inorganic nitrogen. These organisms usually require more than 5 days to become established in a fresh sewage sample but may start promptly in a stream, lake, or effluent sample. Aerobic stabilization of inorganic nitrogen does create an increased oxygen demand; however, attempts to evaluate this parameter according to the following procedures are not valid.

Aerobic stabilization of nitrogen components is becoming increasingly important in impounded waters but is not normally included in the described procedure.

1) Procedure for unchlorinated water

If the sample has been chlorinated, it is recommended that the BOD not be performed. A good job of chlorination renders the BOD meaningless. However, a dechlorination and reseeding procedure is described in the next section for those who desire to attempt it.

Equipment:

burette, graduated in 0.1 ml units with a 50 ml capacity

BOD bottles, 300 ml capacity

Erlenmeyer flask, 250 ml

10 ml measuring pipette

large-tipped volumetric pipette

incubator, controlled at 20° C

Reagents:

Manganous Sulfate Solution: Refer to the procedure for DO.

Alkaline Iodide-Sodium Azide Solution: Refer to the procedure for DO.

Sulfuric Acid: Use concentrated reagent-grade acid (H_2SO_4). Handle carefully, since this material will burn hands and clothes. Rinse affected parts with tap water to prevent injury.

Sodium Thiosulfate Solution: Refer to the procedure for DO.

Starch Solution: Refer to the procedure for DO.

Distilled Water: Water used for solutions and for preparation of the solution water must be of highest quality. It must contain no copper or decomposable organic matter. Ordinary battery distilled water is not good enough.

Phosphate Buffer Solution: Dissolve 8.5 g KH_2PO_4, 21.75 g K_2HPO_4, 33.4 g $Na_2HPO_4 \cdot 7H_2O$ and 1.7 g NH_4Cl in distilled water and make up to 1 liter. The pH buffer should be checked with a pH meter (or pH paper).

Magnesium Sulfate Solution: Dissolve 22.5 g $MgSO_4 \cdot 7H_2O$ in distilled water and make up to 1 liter.

Calcium Chloride Solution: Dissolve 27.5 g anhydrous $CaCl_2$ in distilled water and make up to 1 liter.

Ferric Chloride Solution: Dissolve 0.25 g $FeCl_3 \cdot 6H_2O$ in distilled water and make up to 1 liter.

Dilution Water: Add 1 ml each of phosphate buffer, magnesium sulfate, calcium chloride, and ferric chloride solutions for each liter of distilled water. Store at a temperature as close to 20°C as possible. This water should not show a drop in DO of more than 0.2 mg/l after incubation for 5 days.

Method:

a) The percent dilution to be used must be determined. To make this calculation, one should understand that dilution water at room temperature contains approximately 8 mg/l of dissolved oxygen (DO). Consequently, if the oxygen demand of the sample to be tested is greater than 8 mg/l, dilution of the sample has to be made. It is desirable to have at least 1 mg/l of initial oxygen left after 5-day incubation. Table 1 ia an aid to estimate the dilutions to use.

*Initial DO=7 mg/l				*Initial DO=8 mg/l			
Percent Dilution (%)	Sample added to 300-ml. Bottle (ml)	BOD Range		Percent Dilution (%)	Sample added to 300-ml. Bottle (ml)	BOD Range	
		Min. (mg/l)	Max. (mg/l)			Min. (mg/l)	Max. (mg/l)
1	3	210	490	1	3	240	560
2	6	105	245	2	6	120	280
3	9	70	162	3	9	80	187
4	12	53	123	4	12	60	140
5	15	42	98	5	15	48	112
6	18	35	82	6	18	40	94
7	21	30	70	7	21	34	80
8	24	26	62	8	24	30	70
9	27	24	56	9	27	27	62
10	30	21	49	10	30	24	56
15	45	14	33	15	45	16	37
20	60	11	25	20	60	12	28
25	75	8	20	25	75	9.6	22
50	150	4	10	50	150	4	12

*Initial DO is the concentration of dissolved oxygen in mg/l of the mixture of the dilution water and the sample immediately after initial mixing.

Table 1. An Aid in Selection of Percent Dilution for BOD Determination

Raw sewage usually contains about 100 to 300 mg/l BOD so that 1- and 2-percent dilutions generally are used; settled sewage BOD's usually range from 50 to 200 mg/l, and 2- and 3-percent or 3- and 4-percent dilutions are common; trickling filters use 5- and 10-percent; for activiated sludge effluents, use 10-, 20-, or 50-percent depending upon how good the effluent is. Very strong sewages or industrial wastes are diluted 1 part wastewater to 10 parts dilution water before making the dilutions of 1- to 2-percent. In this way a range of 1,000 to 3,000 mg/l BOD is covered. However, the inexperienced operator is advised not to try to analyze industrial wastes.

b) Fill two 300 ml BOD bottles about half-full with dilution water.

c) Using a large-tipped pipette, measure the precalculated amount of sample into the two 300 ml BOD bottles.

d) Fill each bottle with dilution water and insert stoppers the same way. See that all air bubbles are excluded.

e) Fill two additional bottles with straight dilution water and insert stoppers the same way.

f) Incubate one bottle containing the diluted sample and one bottle containing only dilution water.

g) Determine the initial DO levels of the diluted sample and of the dilution water by running dissolved oxygen determinations on the two remaining bottles.

h) After 5 days, run a dissolved oxygen determination on the incubated bottles. Record the DO contents. (The increase or decrease of DO in the bottles with just dilution water is intended to serve only as a measure of dilution water quality. There should be no increase or decrease more than 0.5 mg/l when compared to the initial DO value of the dilution water.)

Calculations:

BOD values are calculated as follows:

$$\frac{100 \times (\text{Initial DO of diluted sample - DO of sample after 5 days})}{\text{Percent of sample added}}$$

$$= \text{mg/l (5 day BOD)}$$

2) Dechlorination and Reseeding Procedure[3]

Whenever BOD determinations are to be made on chlorinated water samples, sufficient reducing agent must be added to remove the chlorine. After dechlorination, the sample must be "reseeded" with organisms.

Method:

a) Secure an unchlorinated sample of raw sewage or primary effluent about 24 hours prior to the time when you expect to set up dechlorinated and seeded samples for determination of BOD. Collect about one liter of unchlorinated sample and let stand at room temperature overnight. Pour off the clear portion of the sample and use it for the "seed."

b) Check for the presence of chlorine in the composite sample proceeding as follows:

   (1) Carefully measure 100 ml of well-mixed sample into a 250 ml Erlenmeyer flask.

   (2) Add a few crystals of KI to the sample and dissolve the crystals.

   (3) Add 1 ml of concentrated H_2SO_4 and mix well.

   (4) Add five drops of starch.

      1. If no blue color is produced and chlorine is absent, the BOD of the composite may be determined without further treatment. In this case, all of the chlorine has been "used up" by the water and it may be assumed that a sufficient number of organisms remains so that the full BOD will be exerted.

      2. If a blue color is produced, titrate the composite sample with 0.025M $Na_2S_2O_3 \cdot 5H_2O$ to the end-point between the last trace of blue color and a colorless solution. Make the titration very slowly, counting the drops of sodium thiosulfate used and recording the number.

c) To dechlorinate a sample for BOD testing, measure out another 100 ml portion of the well-mixed composite into a clean 250 ml Erlenmeyer flask. Add the number of drops of 0.025M sodium thiosulfate determined necessary for dechlorination in step b4 above. Mix well. Use this sample for determination of BOD. If more sample is needed, place a larger sample into a clean container and add a proportionate number of drops of the sodium thiosulfate for dechlorinating.

d) For seeding of the sample, add 1 ml of the aged seed (step a above) to each of the BOD bottles containing dechlorinated sample.

e) Set up samples of the seed for determination of the BOD using 2, 3, and 4 percent (3, 6, and 9 ml seed) and determine the 5 day depletion due to 1 ml of seed.

Calculations:

If the sample has been dechlorinated and reseeded as described, the 5 day BOD should be calculated as follows:

$$\frac{B - (A + C)}{D} \times 100 = \text{5 day BOD expressed as mg/l}$$

where

A = 5 day DO depletion of seed sample/ml seed
B = Initial DO (mg/l) of diluted sample
C = DO (mg/l) of sample after 5 days
D = Percent of sample used

3) References

1. Water Pollution Control Federation, Simplified Laboratory Procedures for Wastewater Examination, WPCF Publication, No. 18, Washington, D. C., 1968, pp. 38-40.

2. Ibid., pp. 41-43.

5. Interpretation

Aided by natural selection, existing aquatic ecosystems have evolved through geologic time. Organisms have adapted to their environments to the extent that the components of these environments are now the very factors upon which they depend. Deviations from this make-up, especially if sudden, may adversely affect the organisms living there.

Even within a given locale, the environmental conditions which one observes are limitless. Consequently, universal favorable concentrations of dissolved solids, gases, etc., are either exceedingly difficult or impossible to identify. Since toxicity of chemicals varies not only with the types and ages of the organisms concerned but also with duration of exposure, temperature, accompanying dissolved and suspended substances, flow rate, etc., even generalizations concerning concentrations at which specific substances become toxic are not feasible. Because of these difficulties, favorable, tolerable and toxic concentrations are now indicated in this manual on the premise that such information is, at its best, of little significance or, at its worst, misleading.

The following activities are recommended as aids in the interpretation of chemical data (1).

a. Sample the ecosystem periodically over a long period of time. Identify norms and note all biological and chemical changes, especially those which occur suddenly. Evaluate your data in terms of the entire ecosystem. Chemical determinations are of limited significance alone.

b. Determine, in the laboratory, environmental factors which are favorable or tolerable.

c. Use bioassay techniques to identify responses of organisms to various concentrations of potential toxicants and try to determine permissible levels for the ecosystem under study.

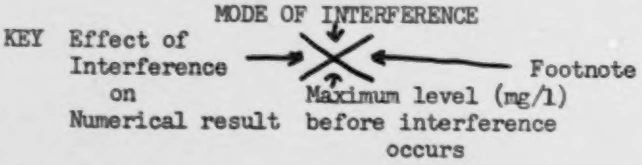
d. Test the laboratory findings in the field to evaluate their validity.

To facilitate interpretation of test results, two tables are included which emphasize those factors which are known to either interfere with chemical tests (Table 1) or influence toxicity (Table 2).

Appendix 1

	Acidity	Alkalinity	Aluminum	Bromide	Chloride	Chlorine	Chromium	Copper	Cyanide	Fluoride	Hardness	Calcium	Magnesium	Hydrogen Sulfide	Iodide	Iron (II)	Iron (III)	Manganous
Acidity			X [e] 1			X [e] 2										X [e] 1		
Alkalinity						X [c] 2						X [c] 3	X [c] 3					
Aluminum						X .5				-X .5		+X .4						
BOD						X [e]												
CO_2	+X [f]		+X [f]				+X [f]	+X [f]						+X [f]		+X [f]		
Chloride				X [d] 5			X 6 10							X [e] 5	X [d] 5		X [e] 5	
Chlorine																	+X [d] .3	+X [d] 11 .01
DO						+X [a] 13										-X [e] 13		
Fluoride		-X 5000	-X .1		+X 7000													
Hardness (EDTA)																		
Ca^{++} (EDTA)		X [c] 20	X 5					X 2									X 20	X 20
Hydrogen sulfide																		
Iron							X [a]	X [b] 5	X [a]									
Mn^{++}					X [a] 10													
Nitrate								-X									X [e]	
Nitrite																	X [c]	
pH (colorimetric)						X [c]											X .04	
Phosphates							-X [b]											
Sulfate																		

KEY



MODE OF INTERFERENCE
Effect of Interference on Numerical result
Footnote
Maximum level (mg/l) before interference occurs

Table 1. - Test Interferences*

Nitrite	pH	Phosphates, Total	Phosphates, Ortho	Phosphates, Poly	Silica	Strontium	Sulfate	Sulfite	Thiosulfate	Ammonia	Turbidity	Temperature	Color	Other
				$-X$				$-X_{10}$						
												X^{g}		X^{a} 4
$+X^{f}$		$+X^{f}$								$+X^{f}$				
	X 8		X^{e}_{25} 7					X 5	X^{a} 7					
$+X^{d}$											X^{b}	X 10	X^{b} 16	$+X$ 9
$+X^{a}_{1}$ 13								$-X^{a}$ 13	$-X^{a}$ 13					
			$+X_{16}$	$+X_{1}$			$+X_{200}$							
	X^{c} 14													
			X^{e}			X^{d}								
								X^{g}_{10}	X^{a}_{10}					
X^{a}		X^{a} 15												
											X^{b}		X^{b}	
$-X^{b}$											X^{b}		X^{b}	
					X_{500}						X^{b}		X^{b}	

Table 1. - Continued

Appendix 1

Footnotes: Table 1.

1. Unhydrolyzed aluminum and/or iron (II) sulfate cause difficulty in determining the end-point. Performance of the titration at boiling temperature alleviates this problem.

2. Free chlorine may be removed by adding 1 drop 0.1M $Na_2S_2O_3 \cdot SA_2O$ to the titration sample.

3. Calcium carbonate and magnesium hydroxide precipitates cause fading end-points and should be removed by filtration.

4. The presence of toxic substances such as heavy metals may interfere with BOD determinations.

5. It also interferes in silver and mercuric nitrate tests.

6. It interferes in mercuric nitrate test.

7. It interferes in silver nitrate test.

8. pH must be in the range of 7-8. Errors are introduced above and below this range.

9. The presence of algae may result in erroneously high Cl_2 determination.

10. Temperature must be controlled at 20°C; otherwise, the Cl_2 concentration will vary.

11. Interference is caused by manganic manganese.

12. Color corrections may be made by using the orthotolidine-arsenite method.

13. Azide modification of Winkler overcomes nitrite interferences. Refer to Standard Methods for additional modifications.

14. pH values greater than 10 favor precipitation of $CaCO_3$, thus causing drifting end-points which may yield low results (EDTA Method).

15. Phosphates do not interfere in the tripyridine method for iron determinations.

16. Periodate method for manganese determinations.

Appendix 1

MODE OF INTERFERENCE Table 1.

- a. 0 interferes with reaction mechanism.
- b. X interferes with phylometric readings.
- c. - interferes with end-point determination.
- d. Test does not differentiate.
- e. It forms interfering ppt under conditions of test.
- f. It disturbs carbon dioxide - carbonate equilibrium.
- g. It alters reaction rate.
- h. It alters concentration.

* The material for this table was obtained from Standard Methods for the Examination of Water and Wastewater. This reference should be consulted for further information.

Table 2. - Interrelationships of Chemical Species Affecting Biological Activity*

	Metallic CN	$CuSO_4$	Pb	Zn	Al	High pH	NH_3	low pH	Cu	High Hardness	Cl_2	$ZnSO_4$	$CdSO_4$	Hg	$NaNO_2$	$NaNO_3$	CN	low DO	Acidity	Temp Inter	CO_2	Low H	Alkalinity	Ca
Acidity	+																							
Alkalinity		−																						
Ca			−	−	−																			
$CO_3^{=}$						+																		
CO_2							−																	
Cl^-																								
Cl_2								+	+															
Cu										−	+	+	+	+	−	−	−	+						
CN^-								+	−									+	+	+				
Low DO			+	+			+		+								+				+			
Metals										−												+		
Fe^{+}_{3}																							+	
Mg																								−

(+) = Synergistic (-) = Antagonistic

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*The relationships depicted in this table were cited in "Water Quality Criteria" by McKee and Wolf. This reference should be consulted for further information.

6. Bibliography

a. American Public Health Association, Standard Methods for the Examination of Water and Wastewater, (13th ed.), American Public Health Association, Inc., New York City, 1971. This includes detailed information concerning the identity, origin, and analysis of numerous chemical parameters. It is an indispensable reference for water pollution studies.

b. Federal Water Pollution Control Administration, Report of the Committee on Water Quality Criteria, Superintendent of Documents, U. S. Government Printing Office, Washington, D. C., 1968. This is a compilation of FWPCA water quality criteria recommendations and supporting information.

c. McKee, J. E.,and H. W. Wolf, Water Quality Criteria, (2nd ed.), Water Quality Control Board, Sacramento, Calif., 1963. Although dated, this is an outstanding compilation of the technical, social, and legal history of water quality criteria. Reviews of Federal and State policies as well as commentaries on an enormous number of chemical pollutants are included. 3827 references are cited.

d. Sawyer, C. N.,and P. L. McCarty, Chemistry for Sanitary Engineers, (2nd ed.), McGraw-Hill Book Co., New York City, 1968. This is a lucid presentation of the theory and methods of sanitation chemistry intended for the reader possessing a solid foundation in elementary chemistry.

Appendix 1

B. Bacteriology

There are numerous types of bacteria and most environments are capable of supporting some bacterial life. Certain bacteria, including those used in food processing or those found in the soil, which enable plants to obtain nutrients, are beneficial. Some bacteria, however, cause food to decay and are responsible for various diseases.

Water can contain many types of bacteria in large numbers Some of these bacteria are harmless to man, but certain types, termed pathogenic, cause diseases such as typhoid fever, dysentary, and cholera. The possible danger of disease dictates that water be free of pathogenic bacteria. Because the cultivation of pathogenic forms is difficult and requires trained personnel, a group of more easily cultivated bacteria is used to indicate the possible presence of pathogens. These indicator organisms include total coliform, fecal coliform, and fecal streptococci organisms.

1. Total Coliforms

Total coliforms include a group of rod or stick-shaped organisms characterized by their ability to ferment a specific sugar (lactose) at 35°C within 48 hours. Although coliforms are introduced to water supplies via water runoff from soil, drains, etc., they are considered significant as indicator organisms because of their predominance in the intestinal tracts of warm-blooded animals. While not all animal wastes contain pathogens, the excrement of diseased animals and animals serving as carriers of pathogens do present health hazards.

The total coliform density is roughly proportional to the amount of excremental waste present. With exceptions, elevated coliform populations are suggestive of significant contamination by excrement of warm-blooded animals. Several factors which cause fluctuations in total coliform populations are summarized in Table B-1.

Table B-1 Factors Influencing Total Coliform Levels

Higher	Lower
1. Sewage intrusion	1. pH changes
2. Nutritive effluents (containing sugar, dairy wastes, etc.)	2. Temperature changes
3. Storm drain overflows	3. Land runoff (prolonged rain)
4. Land runoff (initial storms)	4. Toxic wastes

Coliform population limits have been set bv federal and state health services. These limits vary with the designated use of the water supply and are quite variable within such designations.

Table B-2

Water Quality Criteria with Respect to Total Coliform Populations

Class of Water	Mass.	N.H.	Vt.
a. Drinking water	1 *	1 *	1 *
b. Water of highest quality, designated for ingestion after disinfecting supplies.	50	50	50
c. Suitable for bathing and recreation; irrigation and agricultural uses, good fish habitat; good aesthetic value; acceptable for ingestion filtration and disinfection.	1,000	240	1,000
d. Suitable for recreational boating; irrigation of crops not consumed raw; habitat for wild life and fish; certain industrial cooling and processing use.	Unspecified	--	--
e. Suitable for aesthetic enjoyment, power, navigation, and certain industrial cooling and processing uses.	Unspecified	--	--

*This is a national standard specified by U.S.P.H.S.(1962)

2. Fecal Coliform

Fecal coliform, a component of the total coliform population, is characterized by its ability to reproduce on a special medium (M-FC) at a temperature of 44.5 to 50°C. Because non-fecal coliforms may grow below 44°C and fewer fecal coliforms grow above 45°C, temperature maintenance within the specified tolerance is critical.

Appendix 1

Fecal coliforms are gaining notoriety as pollution indices because of their relatively infrequent occurrence except in association with fecal pollution. Moreover, because survival of the fecal coliform group is shorter in environmental water than for the coliform group as a whole, high fecal coliform levels indicate relatively recent pollution.

When accompanied by fecal streptococci counts, fecal coliform values may aid in the differentiation of animal from human waste (Table B-3). However, caution must be exercised in the interpretation of such data because of technical difficulties in performing precise counts.

Table B-3

Average Individual Density Per Gram of Feces (Indicator Microorganisms From Some Animals)

Animal	Fecal Coliform Million	Fecal Streptococci Million	Ratio Fecal Col./Fecal Strep.
Man	13.0	3.0	4.4
Duck	33.0	54.0	0.4
Sheep	16.0	38.0	0.4
Chicken	1.3	3.4	0.4
Cow	0.23	1.3	0.2
Turkey	0.29	2.8	0.1
Pig	3.3	84.0	0.04

It is anticipated that national standards for water use will be established according to population densities of fecal coliform as shown in Table B-4.

Table B-4

Water Quality Criteria With Respect to Fecal Coliform Population

Kind of Water	Recommended Numbers of Fecal Coliforms
1. Water designated for Primary Contact Recreation	1. Should not exceed a mean of 200/100 ml
2. Water other than for Primary Contact Recreation	2. Should not exceed a mean of 1000/100 ml
3. General Recreational Surface Water	3. Average not to exceed 2000/100 ml

Primary Contact Recreational Activities are defined as those activities in which there is a prolonged and intimate contact with the water involving considerable risk of ingesting water quantities sufficient to pose a significant health hazard.

The Food and Drug Administration recommends that the surface waters above shell fish beds shall not have fecal coliform counts above 70/100 ml.

3. Fecal Streptococci

Fecal streptococcus, as used in this discussion, refers to any streptococcus commonly found in significant numbers in the feces of human or other warm-blooded animals. Fecal streptococci are spherical organisms which generally occur in pairs or short chains when viewed microscopically. They are capable of reproducing at 45°C and, in some instances, at 10°C on a selective medium containing sodium azide and other inhibitors.

Because fecal streptococci do not occur in pure water or virgin soil, their presence in water supplies indicates the existence of warm-blooded animal pollution. Their validity as an index of pollution is enhanced by their inability to reproduce in water supplies. Moreover, fecal streptococci are resistant to salts; therefore, this group could have special value for salt water investigations.

Fecal streptococci determinations, when accompanied by fecal coliform studies, serve as a valuable tool in the differentiation of animal from human wastes (Section 2, Table B-3). In intestinal wastes of human origin, the ratio of number of fecal coliforms to number of fecal streptococci tends to be greater than four. In comparison, when such ratios are determined for intestinal wastes from nonhuman sources, the values tend to be markedly less than 0.7. When interpreting fecal streptococci data, the following three points should be considered.

a. The presence of fecal streptococci in untreated water indicates the presence of fecal pollution by warm-blooded animals.

b. In samples where the source and significance of the coliform group have been questioned, the presence of the streptococcus group should be interpreted as indicating that at least a portion of the coliform group is derived from fecal sources.

c. Because of the uncertainties in die-off rates, the absence of fecal streptococci does not necessarily mean that water is bacteriologically safe.

4. General Procedures

a. Sterilization

It is necessary to sterilize all necessary equipment in order to assure that only bacteria from the collected water sample will be counted. Therefore, the following instructions must be followed closely.

Equipment:

autoclave or pressure cooker

Items for immediate sterilization:

1) for preparation of media (Procedure b)

Petri dishes (number determined by sampling needs)

2) for sample dilution (Procedure e)

1	100-ml graduated cylinder
3	1-ml pipettes
3	125-ml pipettes
3	125-ml flasks

distilled water

3) for filtration (Procedure f)

1-, 5-, 10-ml pipettes (number determined by sampling needs)

Items to be sterilized as needed:

1) for preparation of media (Procedures b and e)

collection of bottles containing 0.2 ml 10% sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$)

2) for preparation of solutions (Procedures c and f)

phosphate buffer in closed container

Method:

1) All glassware and distilled water should be autoclaved. Use 15 lbs. of pressure at 121°C for 15 minutes. The openings of all flasks and cylinders should be wrapped with aluminum foil. Do not autoclave plastic parts or culture media without consulting the manufacturer's instructions.

2) Plastic parts can usually be sterilized by boiling in a water bath for 3 minutes.

3) Filter membranes come presterilized. However, after use, they may be washed off in 95% ethanol, autoclaved at 12 lbs. for 12 minutes for reuse.

   Note: Reuse membranes for the same media only.

4) Petri dishes (Millipore) are presterilized. For reuse, they should be soaked in liquid household bleach for 10 minutes and rinsed thoroughly under running water. Then they should be immersed in 70% isopropyl alcohol for 10 minutes and dried. Following assembly, they may be stored for later use.

b. Preparation of Media

After bacteria are collected and filtered from the water sample, they must be allowed to grow at precise temperatures into visible colonies which can be counted easily. To facilitate growth, the proper nourishment (culture medium) must be provided. Total coliform, fecal coliform, and fecal streptococci require different types of culture media, prepared as follows:

Equipment: (Note glassware need not be sterile.)

3	125-ml flasks
3	100-ml graduated cylinders
2	2-ml pipettes
2	glass stirring rods
1	balance
1	heat source

Appendix 1

Reagents and Media:

Dehydrated M-Coliform Broth (MF Endo Broth)

Dehydrated M-FC Broth Base

Dehydrated M-Enterococcus Agar

Agar

Ethanol (95%)

Rosolic Acid

Sodium Hydroxide

Distilled Water (300 ml)

Procedure:

The following methods describe the preparation of agar media for use without pads. This approach is more convenient for preparation of multiple plates. If absorption pads and broth are preferred, omit the agar and add 2 ml of broth to each pad.

1) Total coliform using M-Coliform or MF Endo broth base

   a) Pipette 2 ml 95% ethanol into a 100-ml graduated cylinder and fill with distilled water to the 100 ml mark. Transfer this to a 125-ml flask.

   b) Add 4.8 g of dehydrated M-Coliform or MF Endo broth base and 1.5 g of agar to the diluted alcohol solution. Mix thoroughly. Note: MF Endo broth base can be purchased with agar already added. If this is done, follow the manufacturer's instructions for preparation.

   c) Cover flask with a foil cap and heat the mixture with agitation until it just begins to boil. (Do not reheat or prolong the heating. This reduces the selectivity of the media.)

   d) Cover the bottom of each sterile petri dish with the broth. This should be done while the broth is still warm. It will gel as it cools, allowing the filter membrane to be placed directly on it. Three or 4 dishes are normally prepared for each water sample (refer to Section e). Petri dishes prepared in this way may be refrigerated for 24 hours.

2) Fecal coliforms using M-FC Broth base

   a) Dissolve 0.8 g NaOH in 50 ml distilled water

   b) Dissolve 0.1 g rosolic acid in 10 ml 0.2M NaOH prepared in Step a.

   c) Place 3.7 g M-FC Broth and 1.5 g agar into a 125-ml flask.

   d) Pipette 1 ml 1% rosolic acid solution (prepared in (b)) into a 100-ml graduated cylinder and add distilled water to the 100 ml mark. Pour this mixture into the flask containing the agar and broth base.

   e) Place a foil cap on the flask and heat, with continuous agitation, to the boiling point. Remove from the heat immediately to avoid destruction of the selectivity of the medium.

   f) Cover the bottom of each sterile petri dish with the warm medium. Dishes prepared according to these instructions may be refrigerated for one week.

3) Fecal streptococcus using M-Enterococcus agar

   a) Add 4.2 g M-Enterococcus agar to 100 ml distilled water.

   b) Heat to boiling. Remove from the heat immediately to avoid destruction of the selectivity of the medium.

   c) Cover the bottom of each sterile petri dish with the warm medium. The prepared dishes may be stored in a cool, dark place for 1 week.

References:

(1) Microbiological Analysis of Water, Millipore Corp., Bedford, Mass., 1969, pp. 3 and 5.

(2) Millipore Experiments in Microbiology, Millipore Corp., Bedford, Mass., 1969, pp. 17-19.

(3) U. S. Department of the Interior, Current Practices in Water Microbiology, U. S. Government Printing Office, Washington, D.C., 1969.

c. Preparation of Solutions

1) Sodium thiosulfate

Chlorine is added to public water supplies and sewage treatment effluents to kill bacteria. When collecting water samples, it is necessary to "deactivate" any chlorine present to avoid killing bacteria after they are trapped in the collection bottle. If this is not done, false data suggesting low or absent quantities of bacteria may be obtained. Sodium thiosulfate is used to "deactivate" the chlorine and may be prepared and stored. When needed, the correct amount of sodium thiosulfate is added to the collection bottle and is autoclaved to assure sterilization.

Equipment:

1 1-ml pipette

1 125-ml flask

250-ml collection bottles (number determined by sampling needs)

metal foil or paper

Reagents:

Sodium Thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$)

Distilled Water

Procedure:

a) Dissolve 10 g $Na_2S_2O_3 \cdot 5H_2O$ in 50 ml distilled water and dilute to 100 ml.

b) Add 0.2 ml of this sodium thiosulfate solution to each 250-ml bottle. If glass stoppered bottles are used, place a thin strip of paper in the neck to avoid "freezing" of the stopper.

c) Seal cap and neck with foil and autoclave using 15 lbs. pressure at 121°C for 15 minutes. After the bottles are sterilized, they should remain sealed until the time of collection. Label each bottle to identify it as sterilized and ready for use.

2) Phosphate buffer

The collected water samples are to be poured through a membrane filter to trap the bacteria in a later step. It is necessary to rinse the funnel with a sterile phosphate buffer solution to assure that all bacteria are washed onto the membrane. The phosphate buffer may be prepared at any time and sterilized when needed for use.

Equipment:

1 500-ml flask

1 1-liter flask

1 1-liter glass bottle (preferably with glass stopper)

2 2-ml pipettes

pH meter or close range pH paper

Reagents:

Potassium Dihydrogen Phosphate (KH_2PO_4)

Sodium Hydroxide

Distilled Water

Method:

a) Dissolve 34.0 g KH_2PO_4 in 250 ml distilled water and dilute to 500 ml.

b) Prepare a 1M NaOH solution by dissolving 4 g NaOH in 50 ml of distilled water and diluting to 100 ml.

c) Add the 1M NaOH drop by drop to the solution of KH_2PO_4 (Step a) until the pH is 7.2. Read the pH using a pH meter.

d) Dilute the adjusted solution to 1 liter with distilled water. Label the solution as "Stock Phosphate" and store for later use.

e) Add 1.25 ml of this phosphate solution to each liter of distilled water being converted to the "working" phosphate buffered water.

f) Autoclave the buffered water in a closed glass bottle using 15 lbs. pressure at 121°C for at least 15 minutes. Be sure to allow the autoclave pressure to drop slowly!

d. Collection of Water Sample

When collecting a water sample, it is important to select a location which is representative of the body of water. The collection of water too close to the shore or within stagnant areas may not yield a representative sample. A thorough analysis involves sample collection at varying depths (i.e., about every three feet). However, analysis for recreation usage requires only that a sample be taken one foot under the surface in the middle of the swimming area. For larger streams or rivers, a sterile device can be lowered from a bridge into the main current and filled. The sample must not be contaminated by surface scum or unnatural turbidity at any time during or after the collection.

Equipment:

250-ml sterilized collection bottles containing 0.2 ml sodium thiosulfate solution (added before sterilization)

Method:

1) Remove the foil hood and paper strip from the stopper of the sterile bottle.

2) Place the entire bottle under the water in an inverted position and turn it upright. Keep your hands clear of the water entering the bottle. In moving water, it is wise to keep your hands downstream relative to the neck.

3) Fill the bottle about 2/3 full and replace the stopper while the entire unit is still submerged. The air space is left in the bottle to allow adequate mixing of the sample later. The sample should be iced immediately after collection. Samples may be held a maximum of 6 hours in the field if necessary, and an additional 2 hours in the laboratory.

e. Filtration Volumes - Selection and Dilution

In using membrane filtration as a means of detecting bacteria in water, definite limitations arise concerning the number of countable bacteria colonies on each membrane filter. If too many colonies develop, some may be fused together making accurate counting impossible. If there are not enough, the count may not be representative. Therefore, certain ranges of colonies on each membrane are used as criteria for selecting the dishes which will give the most accurate representation of the bacterial density population (Table B-5).

Table B-5 Recommended Ranges of Colony Counts for Membrane Filtration

Techniques

Test	Number of Colonies	
	Minimum	Maximum
Total coliform	20	80
Fecal coliform	20	60
Fecal streptococci	20	100
Total bacteria counts	20	200

The selection of sample volumes which result in counts within the above ranges depends upon the actual bacterial population of the sample. A summary of relationships which exist between filtration volume bacterial levels within samples is presented in Table B-6.

Table B-6 Ranges Covered By Representative Filtration Volumes

ml sample	Bacterial count per 100 ml based on			
filtered	20 colonies	60 colonies	80 colonies	100 colonies
100	20	60	80	100
10	200	600	800	1000
1	2000	6000	8000	10,000
0.1	20,000	60,000	80,000	100,000
0.01	200,000	600,000	800,000	1,000,000

As indicated in the Table, a moderately polluted lake containing several thousand organisms/100 ml would require the filtration of at least 1 ml undiluted water.

1) Procedure for selection of filtration volumes

A direct calculation of the filtration volume can be made if there is prior knowledge of the bacterial population density in the water under study. The following relationship is utilized:

$$\text{Sample filtration volume (ml)} = 100 \times \frac{A}{\text{average count/100 ml}}$$

where A = mid-range number of colonies for an acceptable plate count which varies according to the organisms being detected as follows:

A = 50 for total coliform counts

A = 40 for fecal coliform counts

A = 60 for fecal streptococci counts

As a sample problem consider a stream with an estimated total coliform level of 25,000/100 ml. The calculation is: $100 \times \frac{50}{25{,}000 \text{ total coliform/100 ml}}$

thus giving a required filtration volume of 0.2 ml.

To avoid disappointing results, 3 or 4 different volumes should be analyzed to increase the likelihood that at least one membrane will possess an acceptable number of colonies. The following guidelines may aid in the selection of these varying filtration volumes.

a) Total coliform counts should be based on filtration volumes varying by a factor of 4 or less.

b) Fecal coliform counts should be based on filtration volumes varying by a factor of 3 or less.

c) Fecal streptococci counts should be based on filtration volumes varying by a factor of 5 or less.

If no prior bacterial data are available for a body of water under investigation, Table B-7 will be of assistance.

Table B-7 Filtration Volumes for Waters Not Previously Studied

	Filtration Volumes (ml) *		
Source	Total Coliform	Fecal Coliform	Fecal Strep.
Unpolluted Raw Surface Water	1,4,15,60 (33-8000)	1,3,10,30 (67-6000)	1,5,25,100 (20-10,000)
Polluted Raw Surface Water	.02,.08,.15,.5 (4,000-400,000)	.1,.3,1.0,3.0 (670-60,000)	0.1,0.5,2.0 for Animal Pollution 1000-100,000 (0.2,1.0,5.0) or (400-50,000)
Sewage and Dilute Sewage	.0003,.001,.003 (200,000-27,000,000)	.003,.001,.003 (670,000-20,000,000)	0.2x,1x,5x Total Colif. for Animal Pollution 1x,5x,25x Total Colif. otherwise

* Ranges/100 ml covered by the recommended volumes are enclosed in parenthesis.

2) Procedures for dilution

NOTE: Dilution is necessary only if an acceptable count (see Table B-5) was not obtained by using different filtration volumes of the undiluted raw sample. If dilution is necessary, follow the procedures below, being sure to filter the total diluted volume.

The following procedure is for the preparation of a 1:1,000,000 dilution.

To choose the correct dilution, it is convenient to change the filtration volume to scientific notation involving a volume that can be easily pipetted. For example, 0.002 ml can be written as 0.2 ml x 10^{-2}. Then 0.2 ml of a 1:100 (or 10^{-2}) dilution can be pipetted for filtration.

Equipment:

3 1 ml pipettes (sterilized)

1 100 ml graduated cylinder (sterilized)

3 125 ml flasks (sterilized)

distilled water (sterilized)

Method:

a) Place 99 ml sterile distilled water into each of three sterile flasks.

b) Shake the sample bottle thoroughly to assure a uniform distribution of bacteria.

c) Add 1 ml sample water to Flask 1 containing 99 ml sterile distilled water. Mix well. This gives a dilution of 1:100.

d) Remove 1 ml of mixture from Flask 1 and place into Flask 2. Mix well. This gives a 1:10,000 dilution.

e) Remove 1 ml of mixture from Flask 2 and place into Flask 3. Mix well. This gives a 1:1,000,000 dilution.

(For higher dilutions, continue this procedure.)

f. Preparation of Filter for Incubation

Now that the glassware is prepared, the sample collected and diluted, and the petri dishes filled with medium, you are ready to catch and to grow the bacteria. When the water sample is passed through the membrane filter in the filtration apparatus, the bacteria in the water are trapped on the filter.

By removing the filter and placing it in a petri dish prepared with appropriate nutrients, the bacteria are ready for growth in the incubator. Because of the need to transfer the filter from the filtration apparatus to the petri dish, it is essential to follow the aseptic techniques outlined below.

Equipment:

filtration apparatus *

vacuum system (syringe, hand pump and fittings, or standard vacuum pump)

forceps without corrugations on the inside of the tips

methanol or ethanol

gas burner or alcohol lamp

large, wide container for boiling water bath (if filtration apparatus is not presterilized) stand to support (lined) flask over burner

sterilized petri dishes containing nutrients

flasks with diluted samples of water being tested

25-ml graduated cylinder (sterilized) - one for each different water sample

presterilized membrane filters

sponge or cloth

100 ml beaker for soaking forceps

sterilized pipettes (number and capacity is determined by sampling needs)

* This procedure assumes apparatus to be unsterile, but autoclaving in advance is acceptable for Millipore Sterifil Filtration System.

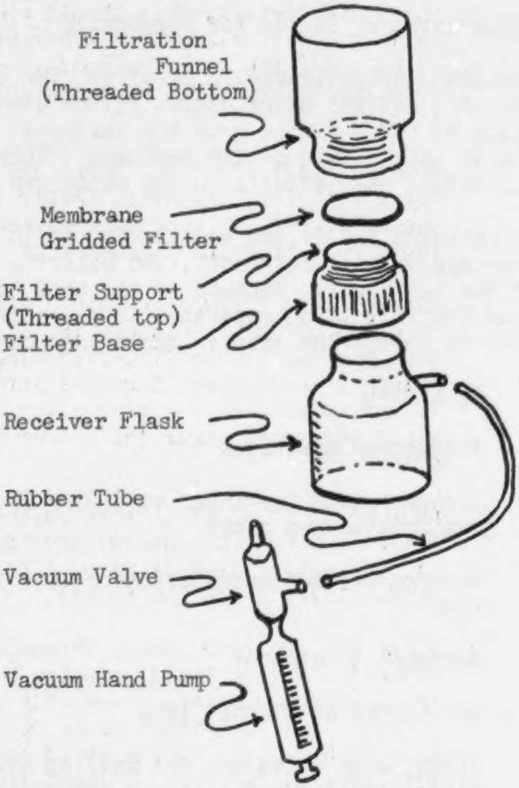

Filtration Funnel (Threaded Bottom)
Membrane Gridded Filter
Filter Support (Threaded top)
Filter Base
Receiver Flask
Rubber Tube
Vacuum Valve
Vacuum Hand Pump

Figure B-1

Method:

1) Set up a boiling water bath by placing a large, wide-mouthed container, containing distilled water, on a stand above a gas burner.

2) Disinfect a laboratory table surface by swabbing it with alcohol. Allow the surface to dry before proceeding. Also swab the bottoms of apparatus to be placed on the table.

3) On the work surface, assemble the funnel unit and receiver flask and connect it to the vacuum system. (Fig. B-1)

4) Set out the petri dishes (raised-lettered side down) and collection bottles.

5) Pour about 20 ml of methanol or ethanol into a small flask and place forceps into the solution. Before using the forceps, they should always be placed briefly in a direct flame from a burner or alcohol lamp to burn off the excess alcohol. Allow them to cool slightly before touching the highly flammable membrane filter.

6) Immerse the filtration funnel and base in the boiling water bath for 3 minutes. After removing them from the water, attach them to the receiver flask. Loosen the funnel to allow placement of the membrane filter.

7) Using flamed forceps, remove a sterile membrane from a sealed package. Discard the blue wax packaging disk and reseal the filter envelope. Place the membrane, grid side up, over the porous plate of the filter support on the filtration apparatus. Carefully place the funnel over the filter base and lock it in place.

8) Pour 20 to 30 ml of sterile buffered water into the funnel. Check for leakage around the funnel base. (If any occurs, repeat Steps 7 and 8.) Leave the buffer solution in the funnel.

9) Shake the prepared sample thoroughly and, using the highest dilution first, pipette the predetermined volume (Procedure e) into the buffer solution in the funnel. (Be sure the pipette is sterilized.) If more than 20 ml of the sample is to be filtered, a sterile graduated cylinder may be used in lieu of the pipette.

10) Reduce the pressure in the receiver flask by creating a partial vacuum in it. Use either a hand pump (e.g., Millipore Vacuum System) or an electric pump.

11) Rinse the funnel by filtering three volumes of 20 to 30 ml of sterile buffered water through the membrane.

12) Loosen the filtration funnel. Remove the cover of a petri dish. Remove the filter membrane from the filter support with flamed forceps. Place the filter membrane, grid side up, on the medium in the petri dish, using a rolling motion to avoid trapping any air.

    Replace the lid on the dish and label it on the bottom, identifying the sample and the filtration volume.

13) Before preparing the next membrane, sterilize the forceps by putting them into the alcohol. Do not forget to flame them. (The rinse with the buffer solution is sufficient to clean the funnel.)

14) Starting with Step 7, repeat the procedures for the remaining sample volumes.

    Note: The buffer rinses (Step 11) clean the funnel sufficiently for all subsequent filtration unless analyzing water samples for drinking purposes.

g. Incubation

As pointed out in "Preparation of Media," bacteria need culture media to grow well. The bacteria also need warmth, moisture, and darkness to grow rapidly. Therefore, the petri dishes containing the bacteria are placed in an incubator to assure proper conditions.

Method:

1) Incubation of total coliforms

After preparation, invert the petri dishes containing total coliform cultures and place them in a standard incubator for 24 hours at 35 ± 0.5°C.

Note: The humidity within the incubator must approach 100%; however, when tightly sealed plastic petri dishes are used, a portion of the broth evaporates, raising the humidity within the dish itself to 100% and making adjustment of the humidity outside the dishes unnecessary. If dish covers are loose fitting, the humidity can be maintained by placing a vegetable crisper containing wet towels in the incubator. The dishes are then placed on top of the towels and covered with the crisper's lid.

2) Incubating fecal coliform cultures

   a) After preparation, invert the petri dishes containing fecal coliform cultures and place them in waterproof plastic bags (3 to 6 dishes per bag).

   b) Submerge the bags in waterbath incubator and incubate at 44.5±0.2°C.

Note: The temperature of the waterbath is critical. Above 44.7°C. counts drop rapidly. Below 44.3°C specificity is lost. Therefore, no more than 20 minutes should elapse between filtration and incubation to prevent nonfecal coliform colonies from developing at lower temperatures. Submergence in waterproof plastic bags reduces the temperature equilibrium time considerably.

3) Incubating fecal streptococcus cultures

After innoculation, invert the petri dishes containing fecal streptococcus cultures. Place them in a standard incubator for 48 hours at 35±0.5°C.

h. Counting Techniques

If all the previous steps were carefully followed, bacterial colonies should now be visible for counting. The results of the count allow us to determine whether the water source is polluted and, if so, how badly.

Method:

1) Counting

   a) Place the petri dish to be counted under the microscope after removing the lid. Lighting for the counts must be from a fluorescent light source as close to directly above the petri dish as possible; the image of the light source is reflected off the colony surfaces into the microscope.

   b) Count colonies in an orderly back-and-forth sweep from top to bottom of the filter, using grids as channels. Be sure to avoid mixing any colonies or counting any colonies twice simply because they are in contact with a grid-line. Count all colonies individually. Even if two or more are in contact, almost invariably, they show a fine line of contact. Other individual colonies may have grown to unusual shapes because of particles or fibres that may have found their way onto the filter membrane. A hand tally is convenient for counting the colonies.

Note: In dishes of total coliform colonies, the coliform colonies demonstrate a greenish metallic luster, or "sheen," which may cover the entire surface of the colony or may appear only in the center of the colony. (Any amount of "sheen" production denotes a coliform colony.) Noncoliform colonies are lighter and do not show this "sheen" even though they may be shiny. In the fecal coliform dishes, the fecal coliform colonies are blue and all other colonies are cream colored (any amount of blue is positive). Fecal streptococcus colonies have a reddish hue, while other colonies range from cream to clear.

2) Calculations:

The count from your membrane must be adjusted for the dilution of the sample and the volume filtered. Results should be in coliforms per 100 ml

Number of Coliform/100 ml

$$= \frac{\text{Number of colonies}}{\text{Vol. of filtration sample}} \times 100$$

For your calculations use the petri dish from the dilution that gives a direct count between 20 and 80. If there are too many colonies on all three dishes, the number is recorded as "T. N. T. C." (too numerous to count). The following shows sample data and calculation:

Sample	Dish #1	Dish #2	Dish #3
No. of coliform	TNTC	39	7
Dilution	1:100	1:10,000	1:1,000,000
Volume filtered	20 ml	20 ml	20 ml

Dish #2 is used for the calculation because the number of colonies was between 20 and 80.

Number of coliform/100 ml

$$= \frac{39}{20\text{x } 1/10{,}000} \times 100 = 1{,}950{,}000/100 \text{ ml}$$

i. Disposal of Cultures

Cultures, whether on filters or in any other medium should be handled with the utmost care, as if they were all potentially dangerous. When you have completed the experiment and observed the results, the cultures should be destroyed or deactivated, and the the petri dishes resterilized. This can be accomplished by the following procedure:

1) Using forceps, carefully remove the petri dish covers and place both covers and dishes into a large beaker or pan containing liquid household bleach for 10 minutes. Unless membranes are to be preserved or reused, they should also be soaked in the bleach.

2) If membranes are to be reused, they should be soaked in ethanol to destroy the colonies. Then sterilization should be completed according to page A-50, step 3.

3) Membranes may be preserved with colonies by drying on a paper towel. They may even be reconstituted with distilled water for later demonstrations.

5. Bibliography

a. American Public Health Association, Standard Methods for the Examination of Water and Wastewater, (13th ed.), American Public Health Association, Inc., New York City, 1971. This is an essential, comprehensive reference for water quality studies, which includes methods of qualitative and quantitative bacterial investigations.

b. Federal Water Pollution Control Administration, Report of the Committee on Water Quality Criteria, U. S. Government Printing Office, Washington, D. C., 1968. It summarizes FWPCA recommendations for water classification categories and criteria and serves as a valuable aid in the interpretation of test results.

c. McKee, J. E., and H. W. Wolf, Water Quality Criteria, (2nd ed.), Water Quality Control Board, Sacramento, Calif., 1963. This is a thoroughly documented coverage of nationwide water quality policies, biological effects of pollutants, and judicial action.

d. Pelczar, M. J., and R. D. Reid, Microbiology, McGraw-Hill Book Co., New York City, 1965. This elementary microbiology textbook includes introductions to the taxonomy, biochemistry, cultivation, control and ecological roles of microorganisms.

e. Microbiological Analysis of Water, Millipore Corp., Bedford, Mass., 1969. A variety of specific techniques for the isolation and identification of bacteria in water samples is presented.

f. Experiments in Microbiology, Millipore Corp., Bedford, Mass., 1969. It contains an illustrated introduction to membrane filtration and culturing techniques and theory and is supplemented by experiments oriented toward the beginning student.

C. Aquatic Biology

A biological investigation of an aquatic community should lead to an understanding of the extent to which it has been affected by man. In order to obtain this understanding, those doing the investigating must be able to assess the biological effects of pollution, to identify organisms, to understand and employ field and laboratory procedures, and to interpret the data which they collect. The following section contains information which will be useful to those conducting a biological investigation.

1. The Basis of the Biological Evaluation of Pollution

Pollutants may affect aquatic environments in two ways. Indirectly, they may produce modifications such as altering the food chain, changing the average annual temperature, or reducing the concentration of dissolved oxygen. Directly, pollutants may act physically or physiologically on the resident organisms.

Biological effects of pollutants may be studied by field observation, laboratory evaluation, or both. In the field, evaluations are usually based on comparisons with an actual or imaginary unpolluted reference or "control" site. For example, the aquatic life downstream from a point of pollution might be compared with that upstream from the source of pollution. Also, the quality of aquatic life in a polluted area may be compared to previous conditions in that area if "prepollution" studies had been made.

Likewise, laboratory evaluation almost always involves a comparison, or control, setup. For instance, the bioassay technique involves the exposing of some type of organism to a series of concentrations of some substance for a stated period of time under controlled conditions.

Qualitative and quantitative evaluations may be used to indicate the "health" of aquatic environments. The indicator concept is based on the idea that there must be some organism which is found only in polluted areas. This is true only for certain types of bacteria which are present in the intestines of warm-blooded aminals. Finding these bacteria demonstrates a strong likelihood that animal excrement is present. Higher forms of life which are pollution-tolerant may also be found in clean water; therefore, finding of these organisms does not necessarily indicate pollution. The species composition of an aquatic community is sensitive to environmental conditions and hence to pollution.

Quantitative data may refer to an entire aquatic community or only to selected or individual taxon. Numbers of individuals, quantity (biomass), or both may be investigated. Rate of production, food web interrelationships, and energy flow might also be investigated.

2. The Identification of Aquatic Organisms

Questions usually posed about an organism which is seen for the first time are "What is it?" and "What is its name?" Because there are over a million and a half kinds (species) of organisms known, a rather elaborate file reference system must be used for naming and classification.

The system of biological nomenclature consists of a series of groupings, or taxa (singular: taxon). Species is the foundation taxon. Similar species are grouped into genera (singular: genus), and similar genera are grouped into families. The system continues in a like manner to order, class, phylum (plural: phyla), and kingdom. Kingdom is thus the broadest, or most inclusive, taxon.

An investigator must make a decision, based upon the purposes for which he wishes to use his data, as to how precisely he wishes to identify the organisms which he observes. For some purposes he might want to know only how many kinds of organisms were present. In this case he could simply designate them as species (a), species (b), species (c), etc. These designations would be based upon careful observations of likenesses and differences of the organisms studied. This type of "classification" would be acceptable for beginning students. If more precision is desired, an investigator would probably make use of an identification key.

The following key to basic types of plankton and small aquatic organisms should enable the student to determine the general type of organism he is observing. He may then wish to proceed to a more complete key. Identification to species level will usually be very difficult and should only be attempted under the guidance of a taxonomist of recognized competence in the particular taxon in question.

Appendix I

Key To Types of Plankton And Other Aquatic Organisms*

Read each question in turn, refer to the specimen for the answer. If the answer is yes, proceed to the paragraph indicated in the "yes" column; if no, turn to the paragraph number in the "no" column. If you have made a mistake, the "no" column reference may send you back to reexamine some earlier decision.

If the answer is "yes," there may be no number given in the "yes" column, but there will be a name in capital letters which is the name of the group of organisms to which the specimen belongs, and a plate number is cited which illustrates one or more examples.

*This Key was prepared by Dr. H. W. Jackson, Training Program, Federal Water Pollution Control Administration, Cincinnati, Ohio.

	Yes	No
1. Is it necessary to use a microscope to see the organism?	3	2
2. Is the organism, or a mass of it, some shade of green or brown? (The shape is probably stringy, round or shapeless.)	9	13
3. Is the body relatively complex, with many tiny active hairs or other external structures and complicated insides?	11	4
4. Do the cells contain internal bodies (usually green or golden brown) called chloroplasts? (Sometimes these cells are contained inside outer covers which may hide them completely or partially. Sometimes they have red "eyespots" or long slender hairs called flagellae.)	6	5
5. Are the cells without any, or at least with very little visible, internal structure? Generally, these cells are bluish-green in color (especially a mass of them together) and are very minute. If so, they are Blue-Green Algae. See Plates I, II.	--	3

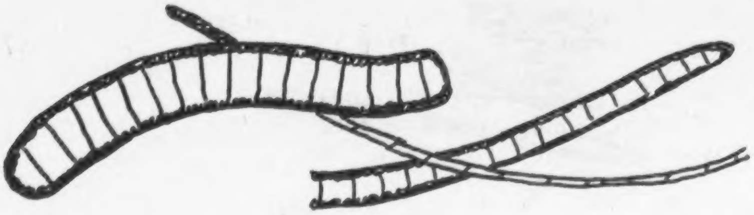
	Yes	No
6. Do these tiny plants consist of single cells or groups of cells which move about by means of one or more long slender hair-like "flagellae?" Red eyespots usually can be seen.* If so, they are Flagellates. See Plates V, VI. *One kind is large and filled with rust-red granules.	--	7
7. Are they golden brown in color, with a tendency to sharply angular edges? They may be cylindrical, thread-like or boat-shaped. Some may move in a hesitant manner. If so, these are Diatoms. See Plates VII, VIII.	--	8
8. These plants are all green. Do they consist of single cells or small clumps of 2 to 4 cells, but not long strings or filaments? (No flagellae or movement should be observed. If it is, return to 6.) These are Coccoid Green Algae. See Plate III.	--	9
9. The following plants (9 and 10) are all filamentous or thread-like, and consist of single cells; cylindrical, barrel-shaped, or roundish, attached end-to-end. Is green pigment (chlorophyll) contained in various shaped bodies within the cells (chloroplasts)? If the mass of filaments appears green or yellow-green to the naked eye, they are Filamentous Green Algae. See Plate IV.	--	10
10. Are the cells of the filament apparently without internal structure (although confused "pseudovacuoles" may sometimes be seen)? Larger oblong cells with heavy walls (heterocysts) or apparently empty cells (akinetes) may occur. Some types with smooth surfaces may move slowly but visibly. Mass of filaments appears some shade of bluish-green or red to the naked		

	Yes	No
eye, occasionally very dark. If as above, these are Filamentous Blue-Green Algae. See Plates I, II.	--	4
11. Is the body composed of a single cell or unit? It may be enclosed in a shell or sheath. It may bear cilia over all or part of the body; flagellated forms may also be encountered: Protozoa. See Plates IXa and IXb.	--	12
12. Is the noncolored body of the organism composed of many cells, with well organized internal structures? There are often one or two crowns of tiny hairs or cilia (which can rarely be seen individually) at one end. One common type crawls like an "inchworm," or "accordion"; others have flattened shells, spines, or other features, but there are no true legs. If as above, this is a Rotifer. See Plate X.	--	13
13. Does the specimen have jointed appendages (joints in the body can also usually be seen), usually with characteristic hairs or setae (may not be extended unless animal is active)?	14	3
14. Is the body completely enclosed in two minute clam-like shells? Jointed legs may be extended from between shells for swimming. These are microcrustaceans, Ostracods. See Plate XII.	--	15
15. Does the elongated, segmented, transparent body (which may range up to approximately 1/2 inch in length) have a head with two eyes? If so, this is a phantom midge larva, an insect: Chaoborus. See Plate XII.	--	16
16. Does the organism have a single eye and two shell-like projections that come down on either side of the legs? Eggs may be present in a large pouch inside the upper back part of the shell.		

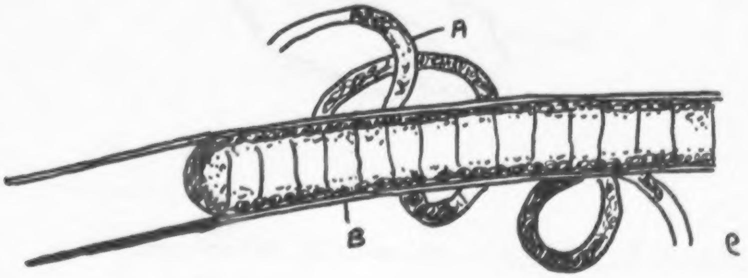
	Yes	No
This is another microcrustacean, a water flea: Cladocera. See Plate XI.	--	17
17. Is there a single eye, the body relatively cylindrical, no side shells, usually tapering toward the rear? There may be segments (or joint) in the body. Two large front "legs" (actually antennae) are used for locomotion. This is another microcrustacean, a Copepod. See Plate XI.	--	18
18. Is the body similar to the above, but roundish or pear-shaped, smaller, and with no segmentation or joints in the body? There are three pairs of relatively large legs. This is the larva of a copepod called a Nauplius. See Plate XI.	--	11

Appendix I

Blue-Green Algae Myxophyceae



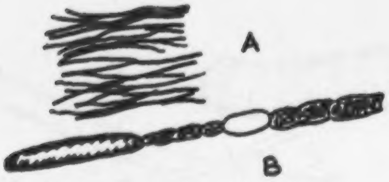
Oscillatoria spp., filaments (trichomes) range from .6 to over 60 µ in diameter. Ubiquitous, pollution tolerant.



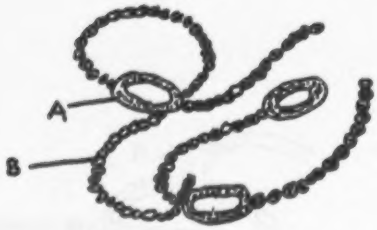
Lyngbya spp., similar to Oscillatoria but has a sheath. A, Lyngbya contorta, reported to be generally intolerant of pollution; B, L. birgei.

Plate I a.

Blue-Green Algae Myxophyceae



Aphanizomenon flos-aquae A, colony; B, filament



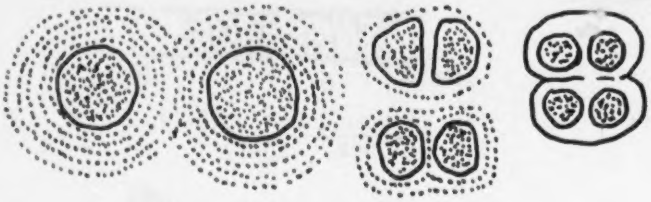
Anabaena flos-aquae A, akinete; B, heterooyst

Plate I b.

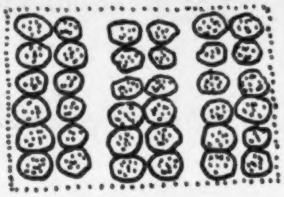
Appendix I

Some Blue-Green Algae

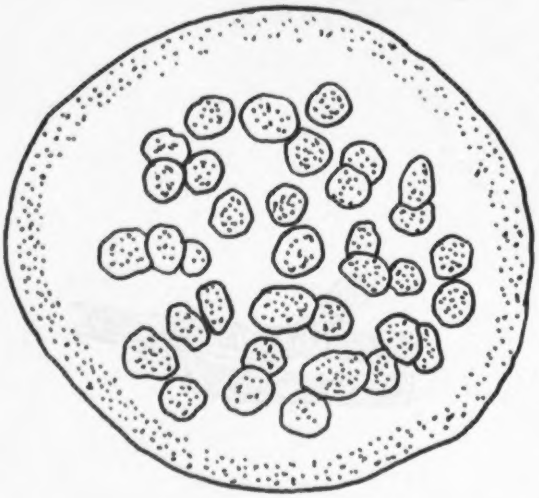
I. Nonfilamentous (coccoid) Blue-Green Algae:



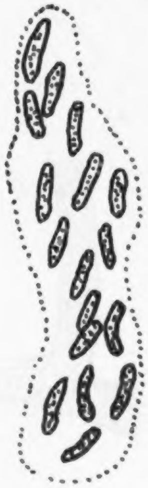
Anacystis (Chroococcus) (X600)



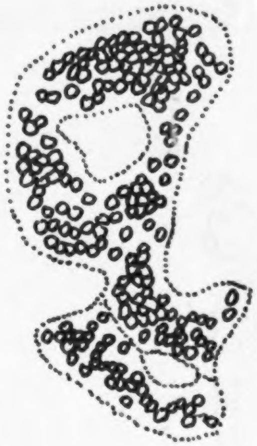
Agmenellum (Merismopedium) (X600)



Coccochloris (Gloeocapsa)(X600)



Microcystis (X600)

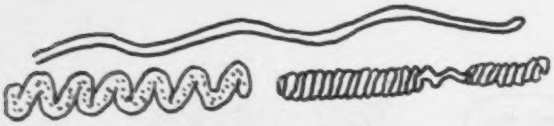


Polycystis (X825)

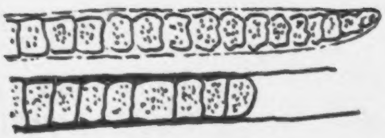
Plate II a.

Some Blue-Green Algae

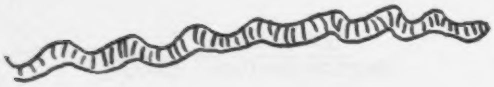
II. Filamentous blue-green algae:



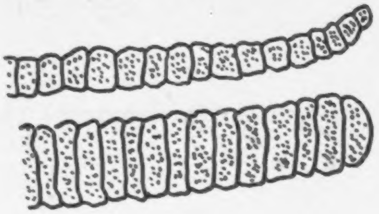
Trichomes of Spirulina. (X600)



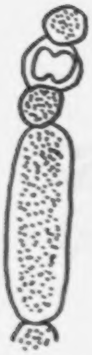
Phormidium (with sheath) (X825)



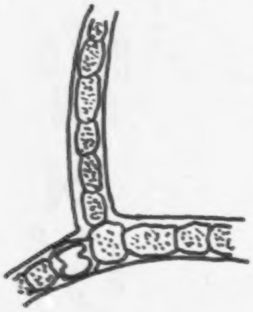
Trichomes of Arthrospira (X600)



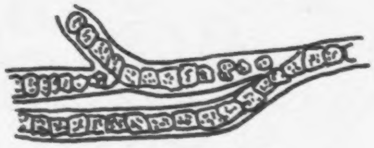
Oscillatoria (without sheath) (X825)



Anabaena (X825)



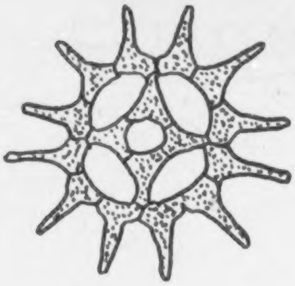
True branching Hapalosiphon (X375)



False branching Tolypothrix (X375)

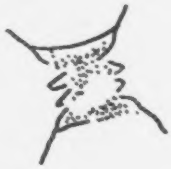
Plate II b.

Nonmotile Green Algae; Coccoid
(Chlorophyceae)

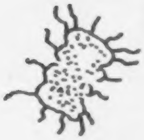


Pediastrum

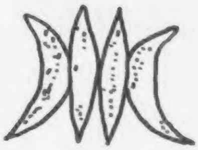
Species of the Genus Scenedesmus



S. caudatus



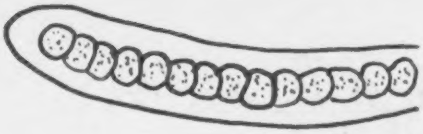
S. abundans



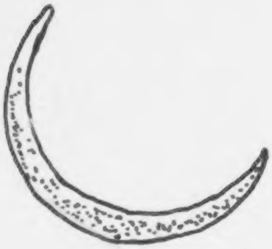
S. dimorphus

Plate III a.

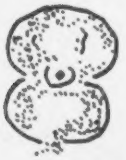
Nonmotile Green Algae: Coccoid
(Chlorophyceae)



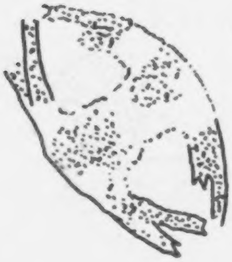
Desmids



Closterium



Cosmarium



Staurastrum

Plate III b.

Nonmotile Green Algae

(Chlorophyceae)

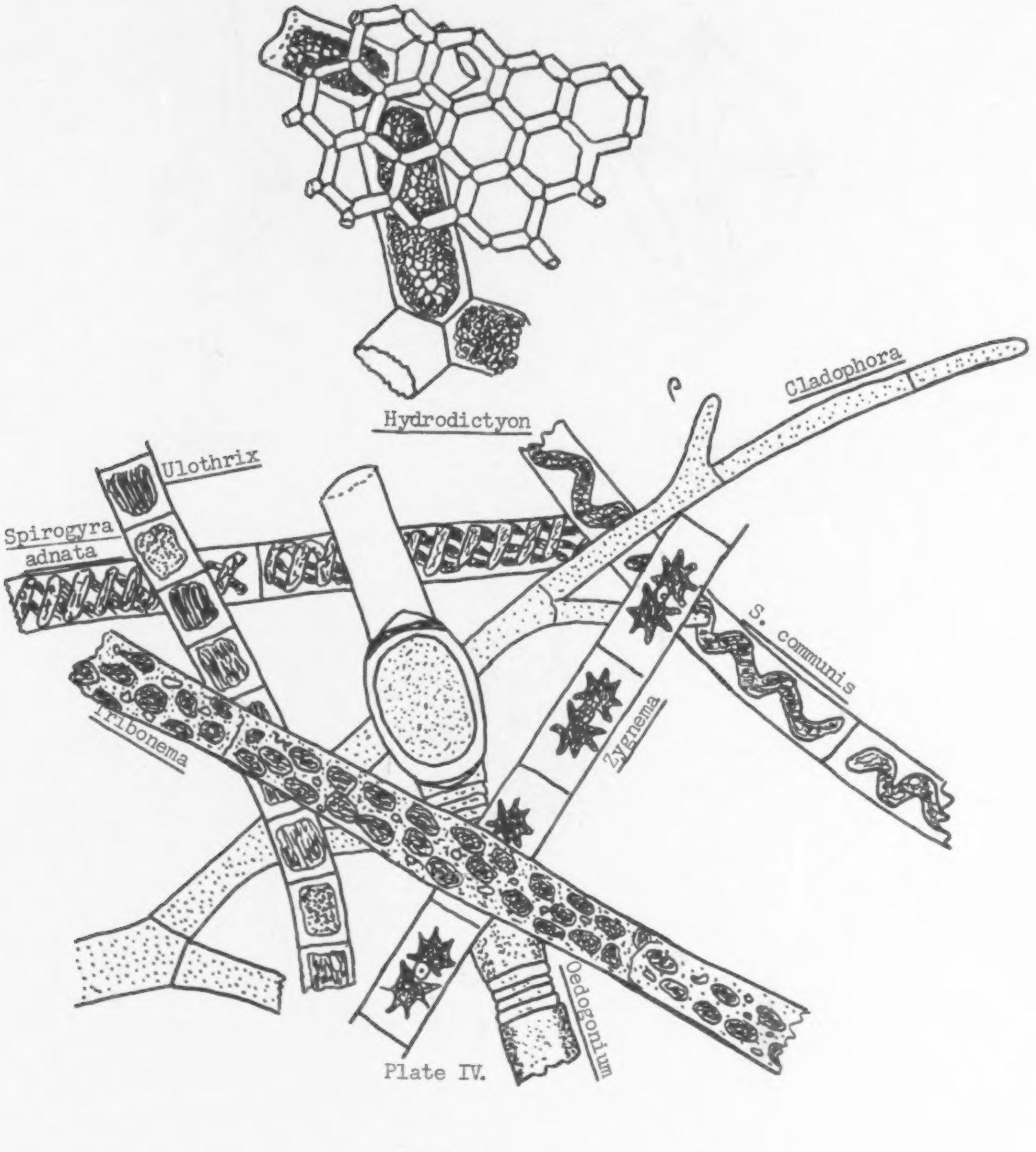
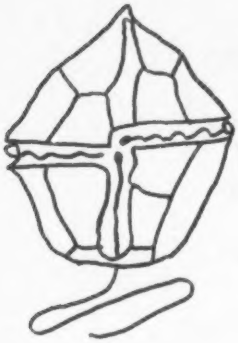
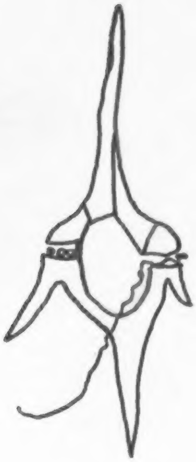

Cladophora
Hydrodictyon
Ulothrix
Spirogyra adnata
S. communis
Zygnema
Tribonema
Oedogonium

Plate IV.

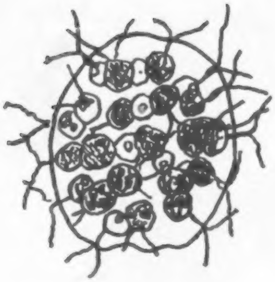
Flagellated Algae



Goniaulax



Ceratium



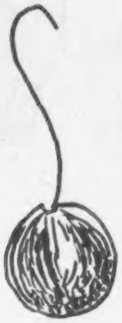
Eudorina

P

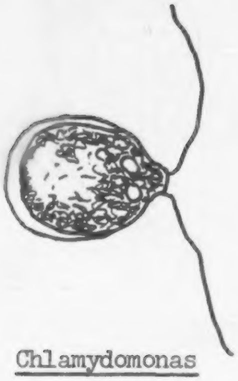
Plate V a.

Appendix I

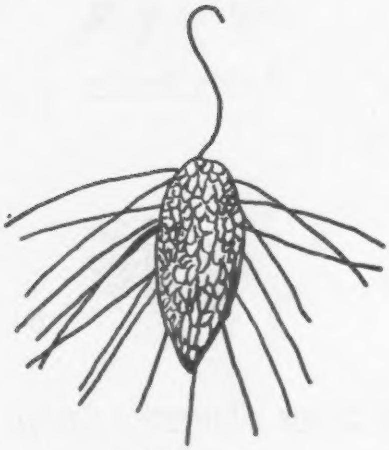
Flagellated Algae



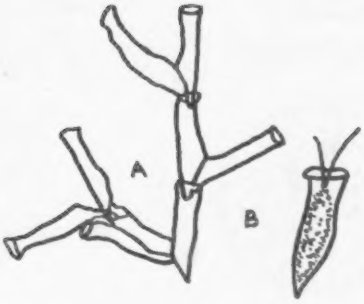
Trachelomonas



Chlamydomonas



Mallomonas



P

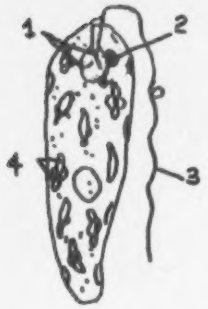
Dinobryon
A, form of colony; B, cell in lorica.

Plate V b.

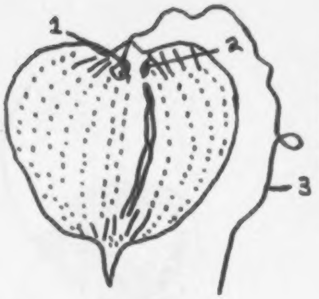
Appendix I

General Morphology Of Algal Flagellates

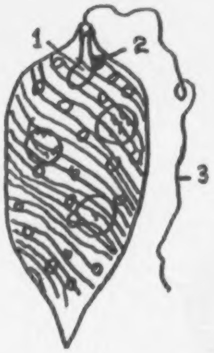
Greens



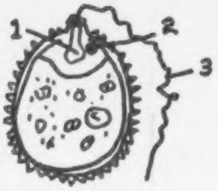
Euglena gracilis



Phacus

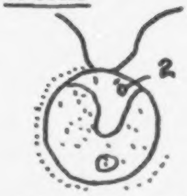


Lepocinclis

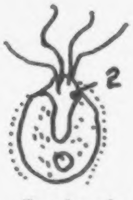


Trachelomonas

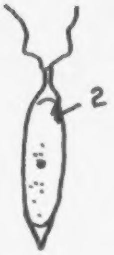
Greens



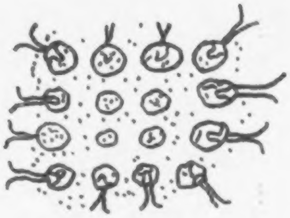
Chlamydomonas



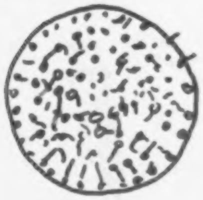
Carteria



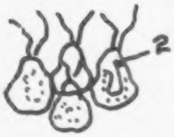
Chlorogonium



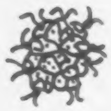
Gonium Sociale



Colony of Volvox



Gonium Pectorale Colony



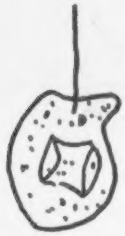
Pandorina Colony

Plate VI a.

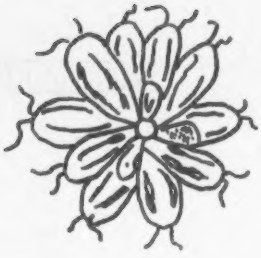
Appendix I

General Morphology of Algal Flagellates

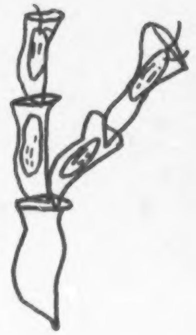
Yellows



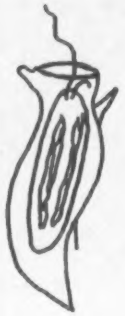
Chromulina



Synura

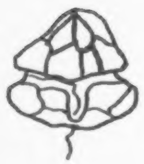


Dinobryon

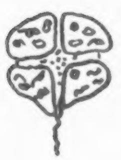


Lorica

Browns



Peridinium



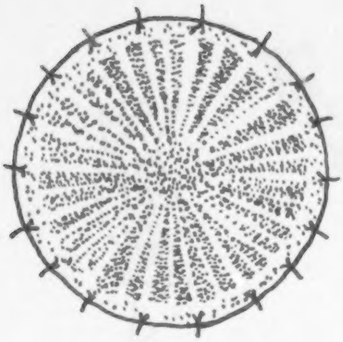
Gymnodinuim

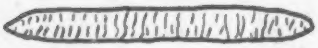
p

Plate VI b.

Appendix I

Diatoms - Bacillariophyceae





Valve views

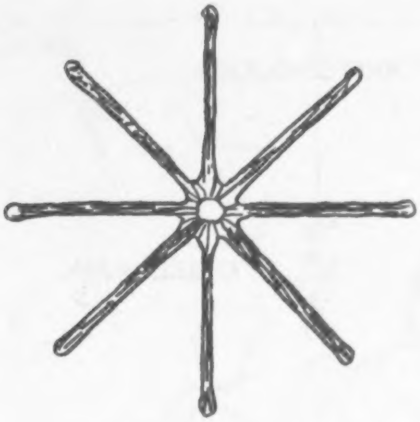
Girdle views,
Stylized to show
basic diatom
structure.

A discoid or central diatom such as Stephanodisous	A pennate or navicular datom such as Synedra

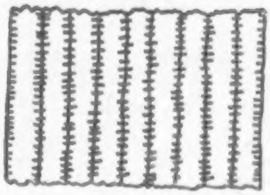
Plate VII a.

Appendix I

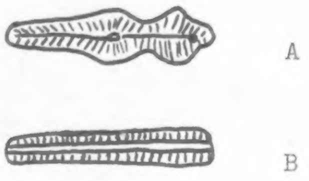
Diatoms - Bacillariophyceae



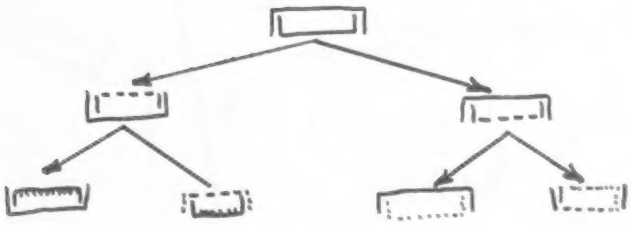
A colony of Asterionella
(girdle views)



A colony of Fragillaria
(girdle views)



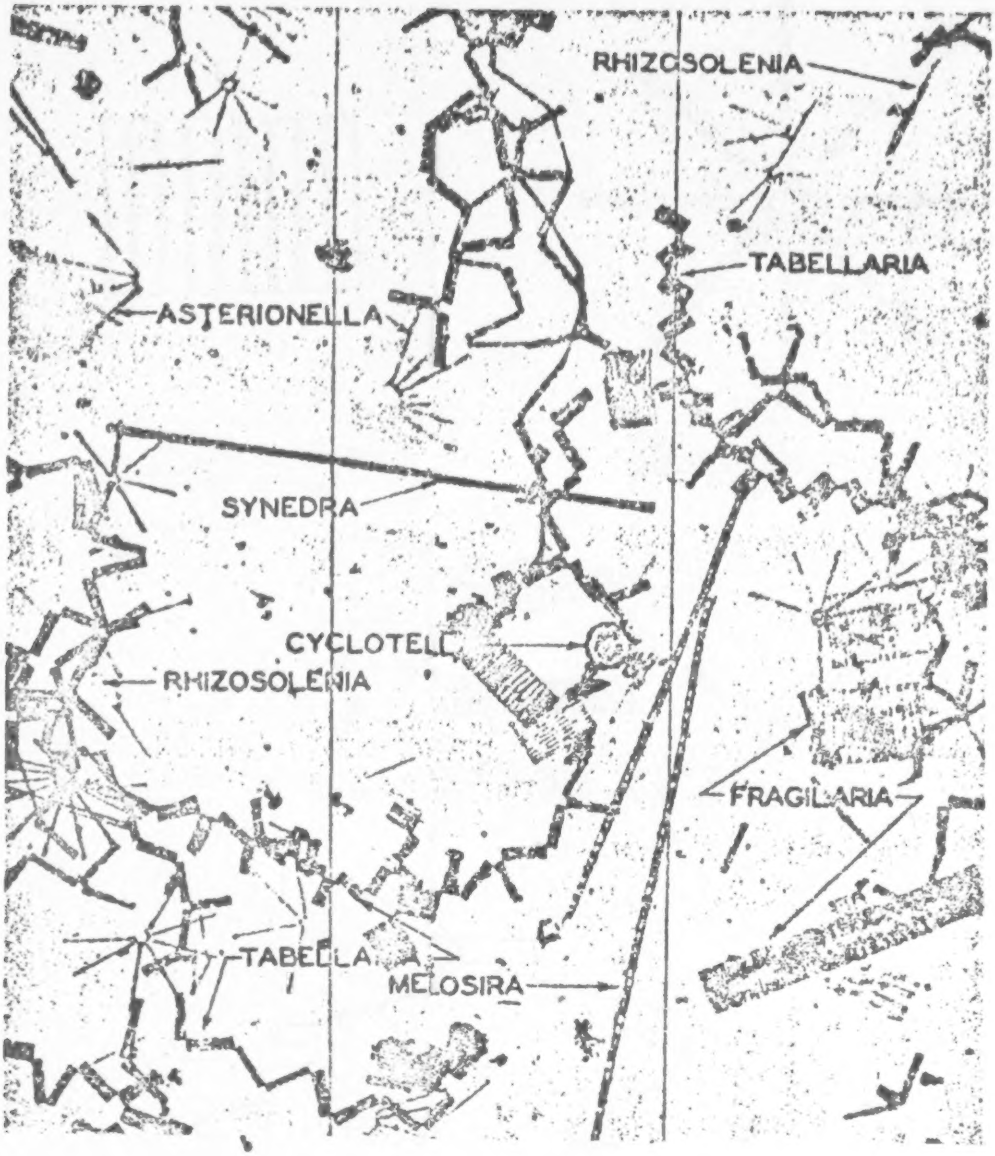
Gomphonoma
A, valve view; B, girdle view.



P

Diagram showing progressive diminution in the size of certain frustules through successive cell generations of a diatom.

Plate VII b.


RHIZOSOLENIA
TABELLARIA
ASTERIONELLA
SYNEDRA
CYCLOTELL
RHIZOSOLENIA
FRAGILARIA
TABELLA
MELOSIRA

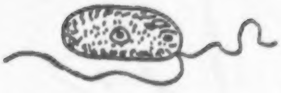
Photomicrograph of Diatoms

Plate VIII.

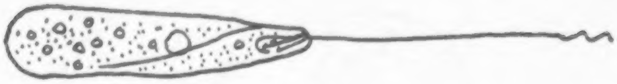
Appendix I

Phylum Protozoa

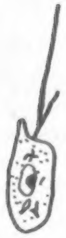
Class Mastigophora, the flagellates



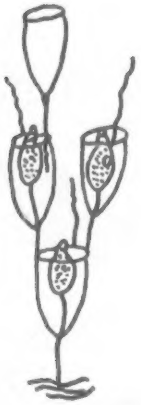
Bodo
Pollution tolerant
19 µ



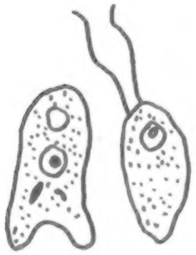
Peranema
(moderately pollution tolerant)
25 µ



Anthophysis
Pollution tolerant
6 µ



Colony of Poteriodendron
Pollution tolerant, 35 µ



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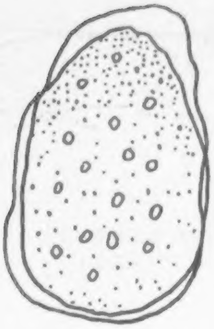
Naegleria
(Pollution tolerant)
10 - 50 µ

Plate IX a.

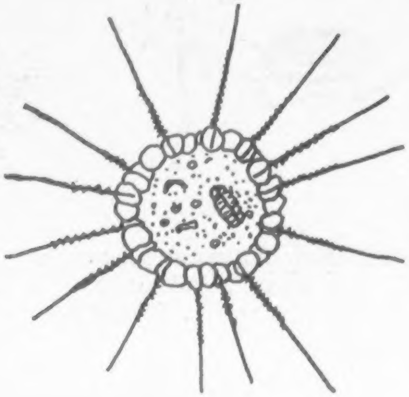
Phylum Protozoa

Class Sarcodina, the amoebas

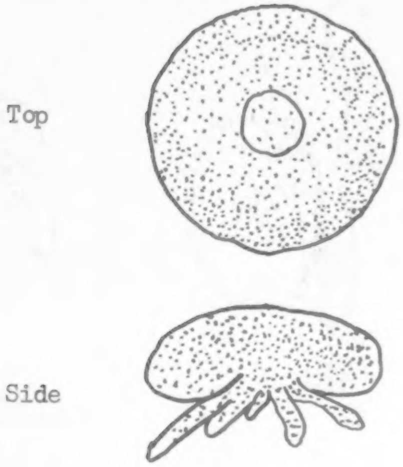
Some forms often found as plankton:



Ameba
45 µ



Actinosphaerium
(Pollution tolerant)
up to 300 µ

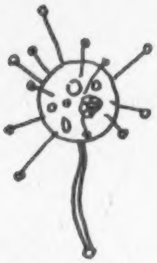


Arcella
(Pollution tolerant)
40 µ

Plate IX b.

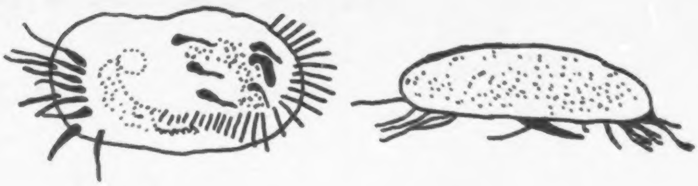
Phylum Protozoa

Class Ciliophora, the ciliates



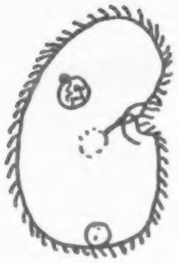
Podophrya, a suctorian ciliate. Pollution tolerant. 20-50 µ

Euplotes
Pollution tolerant, 90 µ

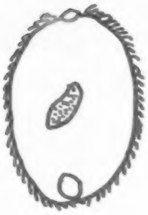


Central View

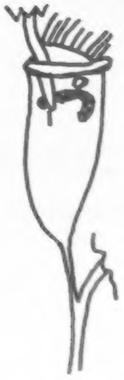
Side View



Colpoda
Pollution tolerant
20-120 µ



Holophrya, reported to be intolerant of pollution, 35 µ



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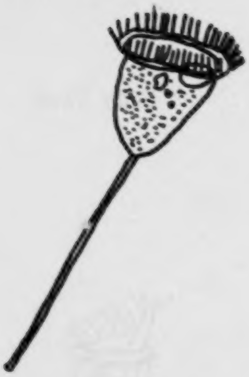
Epistylis, pollution tolerant. Colonies often macroscopic.

Plate IX c.

Phylum Protozoa

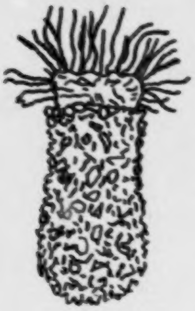
Class Ciliophora, the ciliates

Some forms often found as plankton:



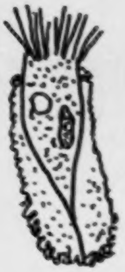
Vorticella

(Pollution tolerant)
Head 75 - 100 μ



Codonella

60 - 70 μ



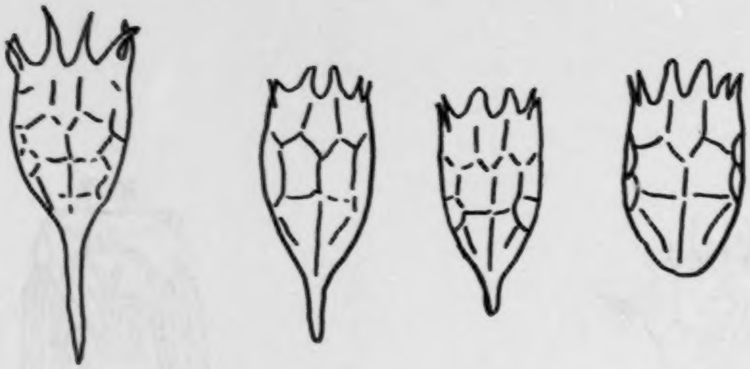
Tintinnidium

100 - 200 μ

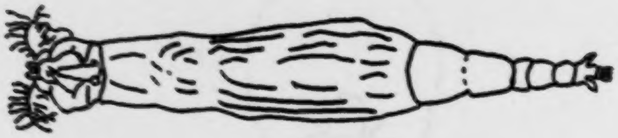
P

Plate IX d.

Planktonic Rotifers



Various Forms of Keratella cochlearis



P

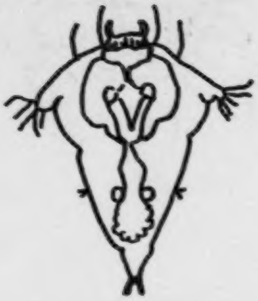
Philodino Rotaria type

Plate X a.

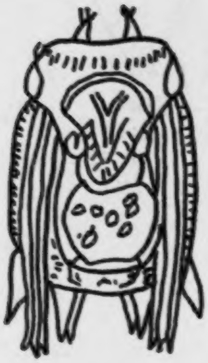
Appendix I

Planktonic Rotifers

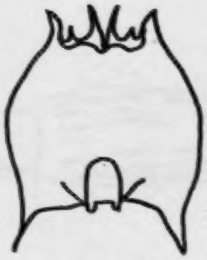
Various Forms of Keratella Cochlearis



Synchaeta



Polygarthra



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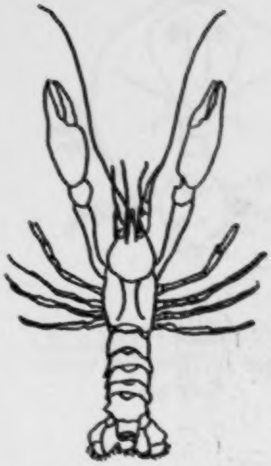
Brachionus

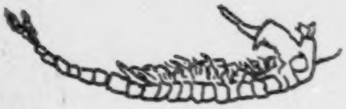
Plate X b.

Appendix I

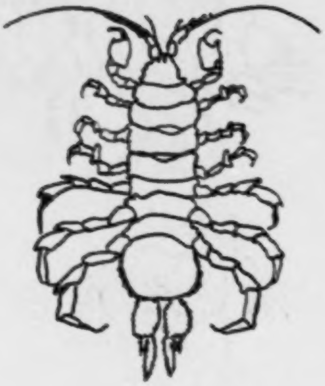
Class Crustacea



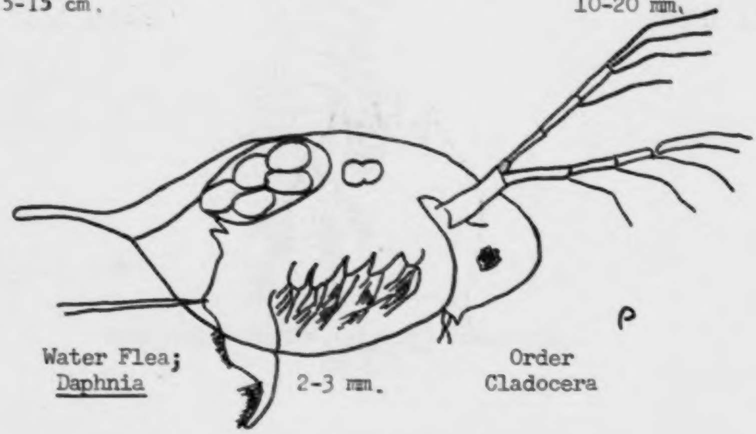
Crayfish, or crawdad; Cambarus, Order Deoapoda
5-15 cm.



Fairy Shrimp;
Eubranchipus, Order
Phyllopoda
20-25 mm.



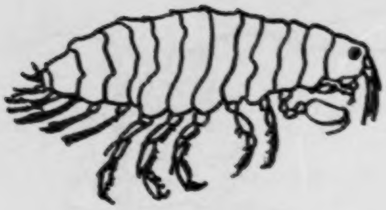
Aquatic Sow Bug; Asellus,
Order Isopoda
10-20 mm.



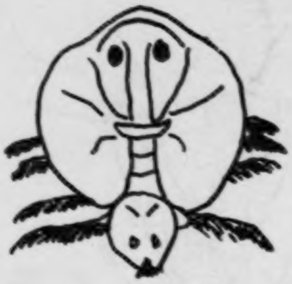
Water Flea;
Daphnia
2-3 mm.
Order
Cladocera

Plate XI a.

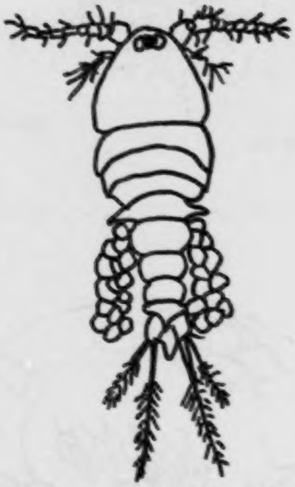
Class Crustacea



Scud; Hyalella,
Order Amphipoda
10-15 mm



Fish Louse; Argulus,
a parasitic Copepod
5-6 mm



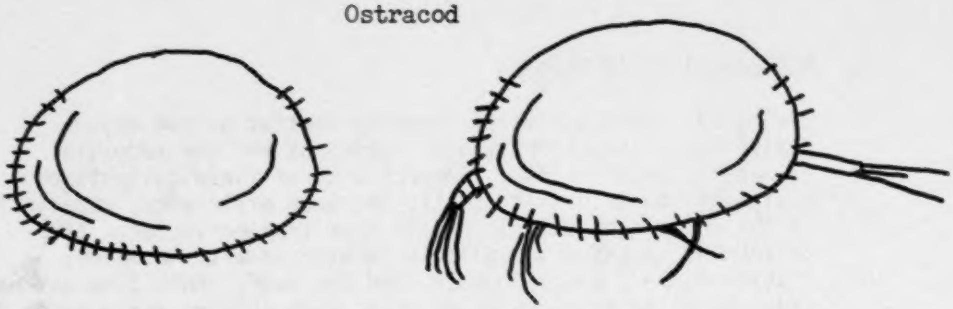
P

Copepod; Cyclops, Order Copopoda
2-3 mm

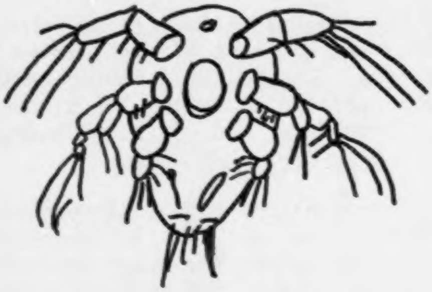
Plate XI b.

Appendix I

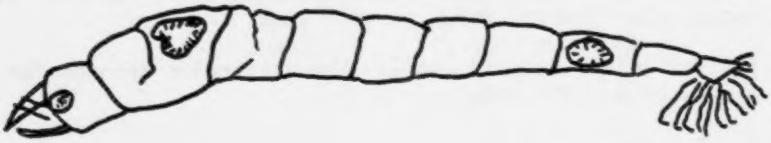
Ostracod



Left: Shell closed Right: Appendages extended



A Nauplius larva of a Copepod



P

Chaoborus midge larva

Plate XII

3. Biological Field Methods

Biological field activities usually consist of two major activities: the collection of specimens and the recording of careful observations. Compact kits of field collecting equipment and materials greatly increase efficiency, especially if the collection site is remote from transportation. All collecting containers should be identified with location, station number, sample number, and the date. Much time may be saved by using data sheets or cards with uniform arrangement for entering the data. The same data sheet may include laboratory or field analysis. Sample data sheets are included at the end of this section. Field notes should be taken in pencil to preserve them in case they get wet.

Observations of the general biological and physical characteristics of the sampling site should be recorded before any sampling is done. Underwater swimming or the use of scuba may be valuable in certain locations for direct observation and collecting. Underwater and aerial photography may be useful.

Because of the diverse nature of aquatic organisms, different methods of collection are used for the various kinds. Aquatic mammals and birds usually require other approaches and are not included. Collection methods for oceanic, estuarine, or freshwater situations are similar. Marine organisms range to larger sizes than those of freshwater. Because of the corrosive nature of sea water, special care should be taken in the design and maintenance of collecting equipment. Site selection and collection schedules for marine sampling are influenced by such factors as tidal currents and salinity distribution rather than river currents, riffles, and pools. Lake collection usually shows less predictable flow patterns. Before going into the field, the investigator should decide on the size range of the organisms to be collected (microscopic, macroscopic) and the kind of organisms (invertebrates, vertebrates, vascular or nonvascular plants) which he will seek.

The following sections explain the collection methods for four groups of aquatic life:

a. Benthos

These are bottom dwelling organisms. They may be attached, crawling, or burrowing forms. Some of the collecting devices are shown in Plates I and II. In most instances, home-made equipment can be substituted for the standard research type. Hand picking of benthic organisms from rocks, sticks, etc., that have been picked out of the water is a fast and much used method for quickly determining what is present and what might be expected in additional samples.

Patches of seaweed and eelgrass and shallow weedy margins are most often studied on a qualitative basis only. The apron net is used for collections in weed beds or in other heavy vegetation. It is simply a pointed wire sieve on a long handle with coarse screening on the top to keep out leaves and sticks. Poking it into and then withdrawing it from the weed masses is the method of operation.

Masses of weeds may be pulled out on the bank (with rakes, grappling hooks, etc.). The benthic organisms can then be observed as they crawl out.

Quantitative estimates of both plants and animals can be made by using a "stove pipe" sampler. This is a hollow tube which is forced down through the weed mass in shallow water and embedded in the bottom. The contents can then be removed and placed into a series of sieves for sorting.

A frame of known dimensions can be placed on the bottom, and the material within is then cropped out. This is especially good for larger plants and for large bivalves. It is also useful on sand and mud flats.

Handle-operated samplers, such as the Jackson, are effective for sampling a variety of bottoms down to the depth of the handles. Such samples are then washed through graded screens to retrieve the organisms.

The Ekman Dredge is a device which is used to collect bottom samples. It should be used in bodies of water which have muddy or sandy bottoms. It will not work well on gravel or rocky bottoms.

The dredge is lowered into the water until it comes to rest on the bottom. In shallow water, you can place it on the bottom; for deeper water, you lower it on a line or a stick. Next the spring mechanism is tripped. This is done by hand in shallow water and by using the messenger (a device which comes with the dredge) in deep water. After the spring is released, the jaws snap shut and enclose the sample. Finally, bring the dredge to the surface and empty the sample into a plastic bag. Refrigerate or cool the bag if the sample will not be studied within an hour. (Benthic organisms decompose rapidly in warm weather.)

Dump the sample out onto the top of a series of graduated mesh, brass screens. The screen with the largest mesh (size of openings) should be on the top and that with the smallest on the bottom. Mesh sizes in between should be arranged in order of decreasing mesh size. Flood the sample with water. (Stirring the sample may be helpful.) This procedure will effect two sortings according to size, "soil" particles and macroinvertebrates. Use forceps to collect the macroinvertebrates. Count, identify, mass, and preserve them.

You may want to compute the density and the biomass of the macroinvertebrates and relate these to other parameters of your study. You may want to study the relationship between "soil" particle size and type and the macroinvertebrates present. An Ekman Dredge can be ordered from Wildlife Supply Company, Saganaw, Michigan and other companies. The cost is about $60.

The Petersen type, which grabs without weights, will take satisfactory samples in firm muds but tends to bury itself in very soft bottoms. It is seldom used in shallow water except as noted below.

The riffle (rift) is one of the most satisfactory habitats for comparing stream conditions at different locations. The hand screen is the simplest and easiest device to use, but the resulting collections are qualitative only. The screen is firmly placed in the stream bed. The upstream bottom is thoroughly disturbed with the feet. The current carries the organisms to the screen. The screen is then lifted, and the contents are dumped into a sorting tray or collecting jar.

The Surber Square Foot Sampler is one of the best quantitative collection devices for rifts. It is firmly planted on the bottom. The stones and other material within the square frame are carefully rubbed by hand to dislodge all benthic organisms. The current carries them into the net. A stiff vegetable brush is often useful, especially if the bottom materials are covered with moss. When bottom materials are picked up which are free from macroinvertebrates, the sampling is finished. Before removing the sampler from the water, the bottom should be "fanned" with the hand to kick up any macroinvertebrates which may have fallen straight down rather than being carried into the net. The organisms are then removed from the net and placed in a plastic bag or a collection bottle. To insure a representative sampling, 3 to 5 square foot samples should be taken at each location.

A Petersen type grab may be used in deep swift riffles. It is placed on the bottom and worked into place with the feet or with poles. After being closed, it is lifted by pulling on the rope in the usual manner.

A strong medium-weight dipnet is the closest thing to a universal collecting tool. Collections are made by sweeping through weeds, over the bottom, or in open water. The handle should be from 4 to 6 feet long and about the weight of a garden rake handle. The rim should be made of steel or brass. The size of the rim stock will depend on the size of the rim; it should be strong but not cumbersome. The netting should be the strongest available preferably with about a 1/16 inch mesh. Nets which are too fine plug up easily and cannot be moved quickly through the water. The net must be protected around the rim. This can be done by sewing canvas, leather, or pieces of old innertube around the rim.

When sampling from vessels, a crane or winch is often used. The general ideas described for shallow water often apply also to deeper waters. The Petersen type grab is probably the best all-around sampler for the greatest variety of bottoms at all depths, from shoreline down to over 10,000 meters. If hauled by hand, the grab should be fitted with 5/8 or 3/4 inch diameter rope in order to provide an adequate hand grip. It is best handled by means of wire ropes and a winch.

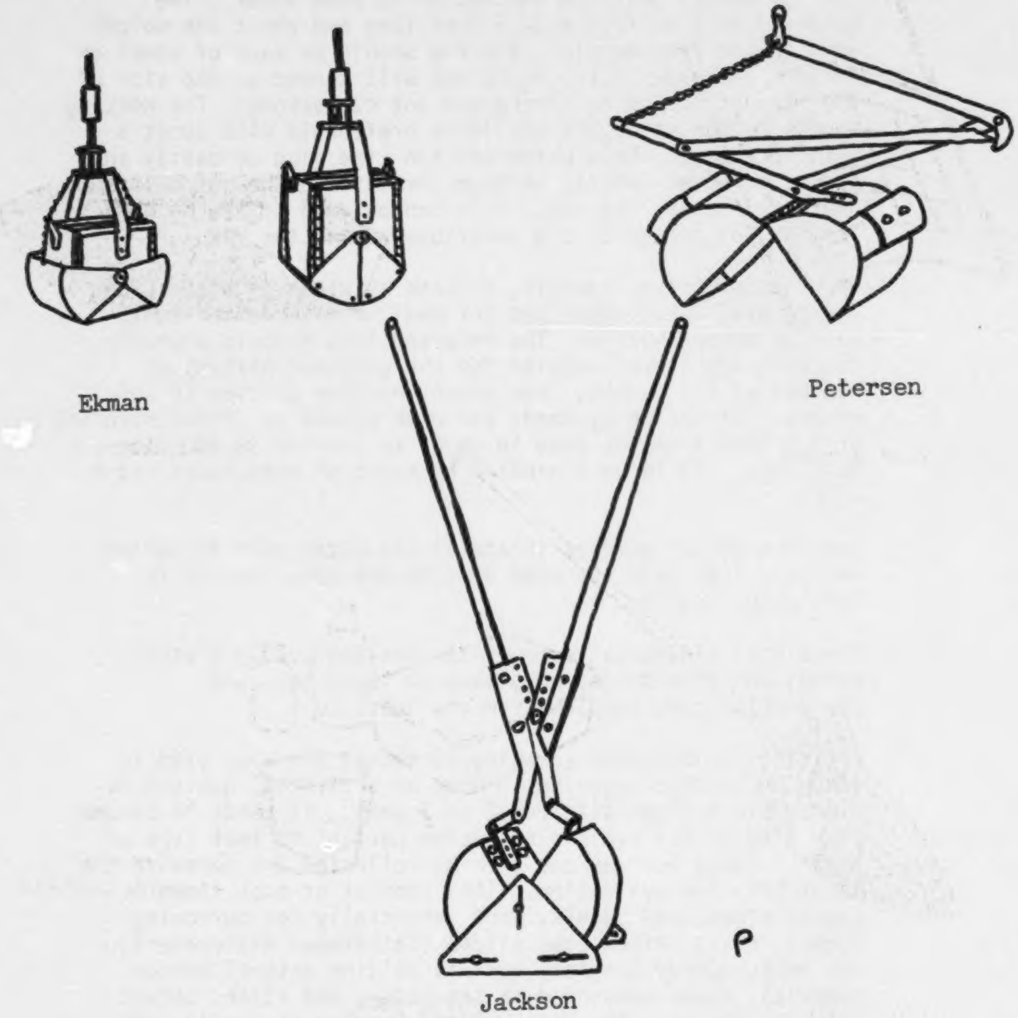
Drag dredges or scrapes (Plate I) are often used in marine waters. They have not been used to any great extent in freshwater studies.

Since most biological communities are not evenly distributed, one should routinely take at least two, and preferably more, samples from any one site.

Artificial substrates (growing surfaces) are also used in studying benthic organisms. When an artificial habitat is exposed in a given site for 2 to 3 weeks, it tends to become populated by all available species partial to that type of habit. These devices can then be collected and taken to the laboratory for evaluation. They consist of such items as cement plates and panels, wood (especially for burrowing forms), glass, microscope slides (Catherwood diatometer), the Hester-Dendy Sampler, baskets holding natural bottom material, ropes suspended in the water, and sticks thrust into the bottom. The Hester-Dendy Sampler is easily made and lends itself well to student use; therefore, it is described in detail below.

The Hester-Dendy Sampler is used to collect benthic (bottom-dwelling) macroinvertebrates. It can also be used to collect attached algae and some types of diatoms. This sampler has .0929 square meter (1 square foot) of exposed surface.

Bottom Grabs

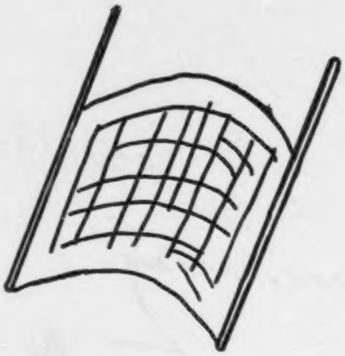

Ekman
Petersen
Jackson

P

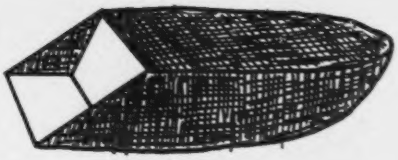
Plate I

Appendix I

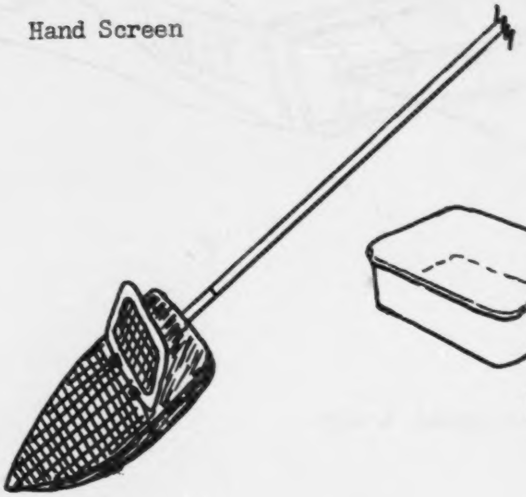
Limnological Equipment



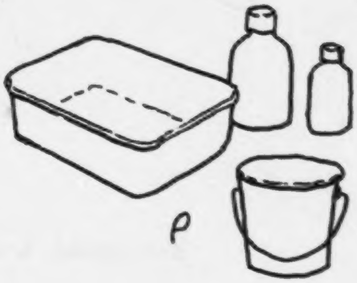
Hand Screen



Surber Sampler



Apron net



Specimen or reagent bottles

Sorting pan

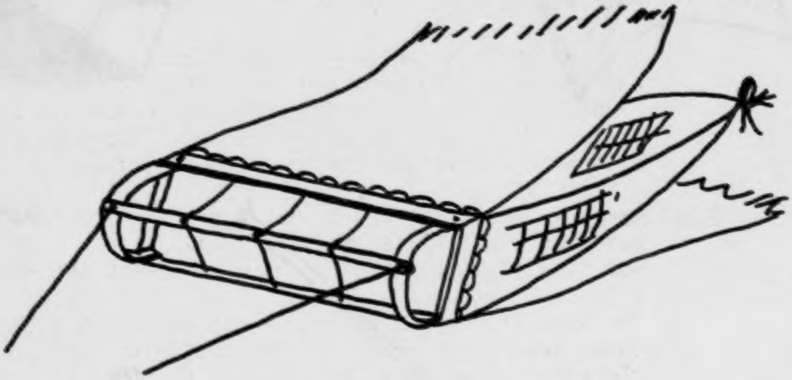
Slurrey bucket

Pail

Plate II

Appendix I

Deep Water Equipment



Biological dredge

Plate III

The materials needed are 1/4 inch and 3/16 inch hardboard, threaded 1/4 inch steel rod, and nuts. Cut the 1/4 inch hardboard into 1 inch squares and the 3/16 inch hardboard into 3 inch squares. Drill 3/8 inch holes in the centers of all the squares.

Assemble the sampler by sliding one of the 3 inch squares on the steel rod, and add one of the 1 inch squares. Continue this procedure until you have added nine more of the 3 inch squares, and eight more of the 1 inch squares. Now add a nut to each end of the steel rod and tighten. A nylon cord may be used in place of the steel rod.

Tighten the nuts until no space remains between the 1 inch and the 3 inch squares and until the squares will stay in place. (An extra 3 inch square may be added without a spacer if you wish to determine which microorganisms might attach themselves to the nonexposed surfaces.)

For any investigation, either place all samplers in flowing water or place all samplers in still waters. (Still waters and flowing waters usually have different benthic populations.)

The samplers may be placed on the bottom if it is composed of sand, gravel, or rock. If the bottom is made of mud, suspend the sampler just off the bottom. (If this is not done, the sampler may become covered with mud and the collected benthic sample will not be representative.)

If the samplers are placed in highly populated or well used areas, they should be hidden so that they will not be disturbed. In most locations, the samplers should be tied to an overhanging branch, to a tree root, or to the bank. Heavy (30 pound test or higher) monofilament fishing line will be less visible than most kinds of string. In very swift water or in locations where attachment is difficult, the sampler may be attached to a metal rod which has been driven into the bottom of the stream or lake.

To show the effects of an effluent on the benthic macroinvertebrates, place samplers upstream and downstream from the point at which the effluent enters. Samplers should be placed on both sides of the stream; for the larger streams and rivers, they should also be placed in the middle.

After the samplers have been in the water for 2 weeks or more, they should be collected. Immediately after removing each sampler from the water, place it in a plastic bag and add some surface water from which the sampler came. This will prevent the loss and drying out of the organisms.

If more than an hour will elapse before you begin to identify the organisms, the plastic bag containing the sampler should be cooled with ice or should be refrigerated. This will prevent the organisms from decomposing, which can happen very rapidly, especially in hot weather.

Open the plastic bag over a white porcelain tray and remove the water and the sampler. Disassemble the sampler and scrape off any macroinvertebrates which are still attached. (A laboratory spatula works well for the scraping.) If large numbers of organisms are present, remove and collect them from one 3 inch square at a time. This will make them easier to count and to identify. (This writer has collected one sampler which had more than 1,300 organisms on it.) The organisms may be preserved in alcohol or formalin for future reference.

The results should be used to compute diversity. The biomass, mass of the life in a specified unit of the environment (1 square foot, in this study), can also be computed. This would give an indication of the productivity of the water.

Benthic collections often consist of large amounts of debris. Various procedures may be followed to separate the organisms from the debris. This separation may be done by hand picking, which is best done on a white enameled tray using light touch limnological forceps. Screening is one of the most practical means of separation. The sample may be dumped onto the screens, and then separated by pouring water over it to wash away the mud and debris. Another method is to place the sample in a bucket or tub and then add water. The mixture is swirled vigorously, and the supernatant is poured through the screen. The residue should be examined for heavier forms which did not float to the top. A variation of this method is to pour a salt or sugar solution into the bucket. The mixture is stirred well, and the supernatant is poured through the screen (save it for reuse). The denser-than-water solution effects the separation of organisms from the debris. A solution of 2-1/2 pounds of table sugar per gallon of water is considered to be optimum for most samples.

Preservation of samples may be achieved by placing them in 80% ethyl alcohol in the field. For prolonged storage, they should be placed in a fresh solution of 70% ethanol. Formalin is also effective in 3% to 10% solutions of the commercial form. Odor and shrinkage problems exist with this preservative. Neutralized formalin eliminates some of the undesirable effects. For short-term preservation, refrigeration and icing are adequate.

b. Periphyton or Aufwuchs

Periphyton is the collection of organisms attached or clinging to stems and leaves of rooted plants or other surfaces projecting above the bottom of an aquatic system. It consists of algae, fungi, small animals, and protists. Periphyton is sometimes referred to as the slime forming organisms.

One qualitative method of collection is to scrape the periphyton from surrounding surfaces, and to place the scrapings in a 4% formaldehyde solution. This can be quantified somewhat by scraping all surface material from a measured area. A more effective quantitative procedure would be to collect the periphyton on an artificial substrate such as glass microscope slides suspended in the water as described above.

By using a microscope and the appropriate identification keys, one can identify the periphyton in the sample.

c. Plankton

Plankton (plancton) is defined as all the microscopic plants, animals, and protists normally swimming or suspended in open water.

A comprehensive plankton sampling program would involve sampling at weekly or more frequent intervals. A year-long study of this type would provide valuable data which could be used to predict conditions in following years.

Phytoplankton (algae) can be collected at the surface in half-liter bottles. For deeper samples, a Kemmerer, Nansen, or other specialized collector may be used. A plankton net is also useful. Sizes number 20 or number 25 are commonly used for collecting phytoplankton. Nets concentrate the organisms in the process of collecting; however, the smaller forms will be lost through any net.

Zooplankton (animals and protists) have the ability to swim away from a collection bottle; so they are best captured with nets which are towed at moderately fast speeds. Number 12 nets (operative size 0.119 mm, 125 meshes per inch) or smaller numbered net sizes are commonly used. The mesh size of the net determines the size of the plankton to be collected.

Both shallow and deep samples are suggested. Shallow samples are taken at a depth of 6 inches to 1 foot. Surface film is also often significant. Deep samples should be taken at as many locations between the surface and the bottom as the study demands. The most complete study would sample the entire water column and would record the kinds of plankton found at each level.

Estuarine plankton should be sampled at different stages of the tide. Since plankton is affected by the forces of winds and currents, a tow is often best made at right angles to the direction of wind or current.

Zooplankton tend to collect near the bottom in daylight and to distribute more evenly at night. One method commonly used to get a representative sample is to take an oblique tow from the bottom to the top of the water column.

Field conditions greatly affect plankton, and they should be carefully noted on the field data card.

Unless the samples will be analyzed within an hour after collection, they should be stabilized in the field. Refrigeration or icing is very helpful, but do not put the ice in the sample. A 5% formalin solution is often used, but it shrinks animals and makes all forms brittle. Lugol's solution is a good preservative. Ultra-violet sterilization is sometimes used to retard the decomposition of plankton. A good methiolate preservative has been developed by the FWQA; it has been described by Weber (1968).

d. Nekton

The larger, free swimming animals such as fish, shrimp, and eels are called nekton. To insure a representative sample, they must be collected from the obscure and unlikely areas as well as the obvious. A check should be made with the local authorities before the sampling is done because many of the standard techniques that are used are not legal for the layman. Professionally trained workers are very important in this area of investigation than perhaps in any other area.

The various devices include haul seines, gillnets, trap nets, traps, trawls, and electrofishing apparatus.

Personal observations by competent personnel and informal inquiries with local residents often yield valuable information. The organized creel census yields data on what kind and how many fish are being caught.

Fish and other nekton are sometimes tagged or branded to trace their movements during migration and at other times.

Miniature radio transmitters can be fed to or attached to them and the nekton can be tracked over considerable distances. Physiological information can be obtained in this manner, also. This is known as telemetry.

e. Sample Data Sheets

Examples of data sheets appear on the following pages. These can be reproduced easily; if desired, they can be punched or stapled into notebooks.

LAKE, IMPOUNDMENT, OR ESTUARY SURVEY

Location and General Characteristics

Reporter: ____________________

1. Name: ____

County: ________________ Township: ________________

Nearest Town: ____

Map agency: __________ Name ____________ No. _____

2. Observed nearby land use: ______________________

____

3. Maximum drawdown or tidal range ________________

4. Depth: Average ____________ Maximum ____________

5. Area: ________________ Shoreline Length _________

6. Shoreline development*: ________________________

7. Watershed size: _______________________________

8. Nature and extent of erosion observed: _________

____

9. Possible pollution sources: ____________________

____

10. Study Station No. :________ Description: _________

____

____

____

____

____

____

$$* \frac{S}{2\sqrt{a\pi}}$$

S = Shoreline length
a = area

L-1

GENERAL MAP

Showing station locations

LAKE, IMPOUNDMENT, OR ESTUARY SURVEY

Present Conditions

Station: ____________ Date: __________ Time: _______

1. Weather

   a. Cloud cover: ______________________________
   b. Wind direction: __________ Velocity: __________
   c. Air Temp: ______________ Precip: ___________
   d. Other: __________________________________

2. Waves

   a. Height: ______ Length: _______ Fetch: _______
   b. Other: __________________________________

3. Ice, thickness: ____________ Condition: ________

   ____

4. Floating materials: ___________________________

   ____

5. Water Color: ________________________________

6. Nature and origin of color: ___________________

   ____

7. Odor, if distinctive: __________________________

8. Secchi disc: _________________________________
   or
   Turbidity rod: _______________________________

9. Notes:

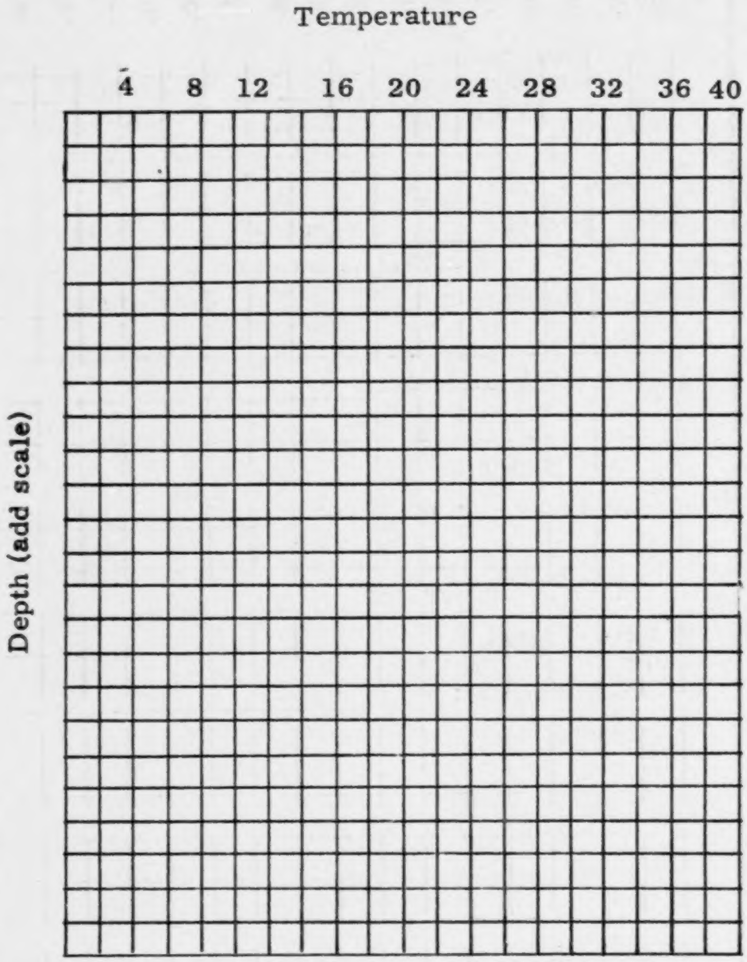
   ____
   ____
   ____
   ____
   ____
   ____

L-2

LAKE, IMPOUNDMENT, OR ESTUARY SURVEY

Temperature Profile

Station: ________________ Date: __________ Time: _____



LAKE, IMPOUNDMENT, OR ESTUARY SURVEY

Physical and Chemical

Station: ____________ Date: ____________ Time: ____________

	Depth	DO mg/l	Other Determinations (note method used)
a.			
b.			
c.			
d.			
e.			
f.			
g.			
h.			
i.			
j.			
k.			
l.			
m.			
n.			
o.			
p.			

L-3b

LAKE, IMPOUNDMENT, OR ESTUARY SURVEY

Physical and Chemical

Station: __________ Date: _________ Time: ________

Other Determinations (note method used)

a. __________

b. __________

c. __________

d. __________

e. __________

f. __________

g. __________

h. __________

i. __________

j. __________

k. __________

l. __________

m. __________

n. __________

o. __________

p. __________

L-

LAKE, IMPOUNDMENT, OR ESTUARY SURVEY

Biological Data - Plankton

Station: __________ Date: ________ Time: ________

Survey Counts

Sample No.: _____ Type: _________ Depth: ________

Procedure: ______________________________

Results: ________________________________

____

____

____

____

Sample No.: _____ Type: _________ Depth: ________

Procedure: ______________________________

Results: ________________________________

____

____

____

____

L-4a

LAKE, IMPOUNDMENT, OR ESTUARY SURVEY

Biological Data - Plankton

Station: ________ Date: ________ Time: ________

Qualitative, Differential, or Proportional Counts

L-4b

LAKE, IMPOUNDMENT, OR ESTUARY SURVEY

Biological Data - Fish

Station: ____________ Date: __________ Time: __________

How collected: ________________________________

Sample No.	Σ Weight	No. of fish
________	________	________

Dominant Kinds

________________________________ __________

________________________________ __________

________________________________ __________

________________________________ __________

Other: ________________________________

____

Sample No.	Σ Weight	No. of fish
________	________	________

Dominant Kinds

________________________________ __________

________________________________ __________

________________________________ __________

________________________________ __________

Other: ________________________________

____

L-5

Appendix I

AQUATIC VEGETATION MAP

LAKE, IMPOUNDMENT, OR ESTUARY SURVEY

Biological Data - Bottom Forms (Benthos and Periphyton)

Station: ________________ Date: ________________

1. Aquatic Vegetation

Kinds of Plants	Extent of Coverage

How collected: ________________

Nature of bottom: ________________

2. Periphyton

Kinds	Extent of Coverage

Description:

____

____

How collected: ________________

Nature of bottom: ________________

3. Attached Algae

Kinds	Extent of Coverage

How collected: ________________

Nature of bottom: ________________

L-6

BOTTOM TYPES MAP

LAKE, IMPOUNDMENT, OR ESTUARY SURVEY

Biological Data - Bottom Forms (Benthos and Periphyton)

Station: __________ Date: ________ Time: ________

Sample No.	Unit	No. Spp.	No. Ind.

1. Insects		2. Other Invertebrates	
Kinds	No. or Rel. Abundance*	Kinds	No. or Rel. Abundance*

How collected: ______________________________

Nature of bottom: ______________________________

* + = present	a = abundant
c = common	d = dominating

L-7

LAKE, IMPOUNDMENT OR ESTUARY SURVEY

Bacteriological Data

Station: ____________ Date: __________ Time: ________

How and where collected: ________________________

____

____

Time collected: _____ a.m. / p.m. Temp: ____________ °C

Collected by: ________________________________

Tests started _______ a.m. / p.m. By: ______________

Tests requested: (check)

Coliform: ______________

Fecal Coliform: ______________

Fecal Streptococcus: __________

Remarks: ______________________________________

____

____

____

____

____

____

____

____

Test results:

Coliforms: ____________ 100 ml.

Fecal Coliforms: __________ 100 ml.

Fecal Streptococci: _________ 100 ml.

Method: Membrane Filter ( )

L-8

NOTES

4. Biological Laboratory Methods

Living specimens are the most desirable for laboratory analysis. Unfortunately, the investigator must often work with preserved specimens. When conducting a comprehensive investigation, a biologist usually collects samples faster than he can analyze them.

After the laboratory data have been recorded, they should be interpreted with reference to the field notes which were taken at the time of sample collection.

a. Benthos

Although some fish (i.e., flounders) are listed as benthic organisms, the usual bottom sample is analyzed for the macroinvertebrates which it contains. After the foreign material has been removed from the sample (see Section 3), the investigator counts, identifies, and determines the mass of the organisms.

A technique commonly used is to pour the sample into a white porcelain tray which has some tap water in it. Laboratory forceps are usually used to remove the organisms. If the sample contains large numbers of organisms, the investigator will find a mechanical hand counter very useful. The organisms should be placed on blotting paper for 1 minute before the mass determination is made.

If the investigator does not wish to "key out" the organisms, he may simply sort and group on the basis of like appearance. He might sketch and/or describe the different kinds and report something like "75 individuals of taxon (or type) 1."

b. Periphyton or Aufwuchs

Direct analysis of the growths attached to the substrate can be carried out but must be restricted to the larger organisms. This is due to the difficulty of keeping the material in an acceptable condition under the short working distances of the objective lenses of compound microscopes and due to the fact that transmitted light is not adequate when the colonial growths are thick or the substrate is opaque.

More often the periphyton is scraped from the substrate and then processed. An aliquot part of the sample may be counted using methods frequently employed in plankton analysis. The number of organisms per unit of volume can then be determined.

The total dry weight of the scrapings and the ash-free dry weight (which eliminates inorganic sediments) can be determined and compared. A packed biomass and volume could be determined by centrifugation of the scrapings. Nutrient analyses serve as indices of the biomass by measuring the quantity of nutrient incorporated. Total organic carbon, carbon equivalents (COD), and organic nitrogen determination would be helpful. Phosphorus has limitations because cells can store excess above immediate needs. Chlorophyll and other bio-pigment extractions might be carried out to determine the amount of these which are present. Other investigations might measure carbon-14 uptake, oxygen production, or respiratory oxygen demand.

Qualitative studies would produce such results as the kinds found, ratios for number of individuals per kind found, and a frequency distribution of varieties found.

Quantitative investigations could yield amounts per unit area, milligrams per square centimeter. Rate studies could determine such things as milligrams per day of biomass accumulation or milligrams of oxygen produced per milligram of growth per hour.

c. Plankton

Microscopic examination is most frequently done in the laboratory to determine the number and kinds of organisms present. Optical equipment need not be elaborate for qualitative studies. If more precision is required, such items as a Whipple counting eyepiece, a mechanical stage, and a stage micrometer may be used.

Precision-made counting chambers such as Sedgewick-Rafter counting cells, Palmer-Maloney counting cells, or haemocytometers are required for quantitative work with liquid mounts. Qualitative "counts" are lists of the kinds of organisms found and the numbers of each per unit of volume or area.

The organisms are observed and, by means of a suitable series of multiplier factors, projected to a number or mass per unit volume. Counting of an unconcentrated sample eliminates manipulation. If the density of organisms is low, more area can be examined or the sample can be concentrated. The concentration of the sample provides more organisms for observation, but this introduces additional errors and takes more time.

Several methods of counting are in general use. The numerical or clump count is regarded as the simplest. The areal standard unit method (See Standard Methods) provides more information. The cubic standard unit method is a logical extension of the areal method, but it has not achieved wide acceptance due to its difficulty.

The field count is done by counting and tallying all individuals of each type present in a field of view. A good way to do this is to list the most common types separately, record their counts, and enumerate the other forms present. This is done for five or ten randomly chosen fields. Finally, the results are tallied and the percentage of each type is computed.

The Five Hundred Count is done by moving the slide at random and counting and tallying all the types until a total of 500 cells or clumps have been counted. Then the investigator should tally the results and compute the percentage of each type as before.

Sometimes, measurements are made by means other than microscopic counts. Settled volume of killed plankton may be measured in an Imhoff cone or a graduated cylinder after a standard period of time. This will evaluate only larger forms. A gravimetric method involves drying at 60° C for 24 hours followed by ashing at 600° C for 30 minutes. This is particularly useful for chemical and radiological analyses.

Chlorophyll can be extracted by filtering, drying for 24 hours, and extraction with methyl alcohol. Evaluation can be made by using a colorimeter or by using chromatographic methods. A membrane filter may be used. The filter can be cleared with immersion oil and organisms can be observed directly after 24 hours, or the collected material can be washed off and observed immediately.

d. Nekton

Population studies are often done with the larger animals. The individuals should be checked for general condition and for the presence of parasites.

As mentioned before, the collection of fish is best done by professionals.

e. Bioassays and Biomonitoring

The bioassay technique may be used on any appropriate organisms from protozoa to fish. In the lab, two types of apparatus, the static jar and the continuous flow, may be employed to provide the various dilutions of the toxicant used.

Static jar tests are containers with a known concentration of substance and organisms. These tests are seldom run for more than 1 week and are read only in terms of percent survival or kill. This is usually termed acute toxicity.

The continuous flow apparatus may also be used to measure acute toxicity. This setup allows a solution to flow into the containers at certain time intervals. Continuous flow apparatus is virtually essential for long-term tests at sublethal concentrations. Parameters other than lethal thresholds can then be measured, such as the effect on growth rate and breeding success of a species of fish.

Biomonitoring permits continuous surveillance over the toxicity of an effluent. This technique involves the placing of living organisms in test waters. A normal length of time to run the test in water pollution studies is 96 hours. For the results to be significant, a 50% or greater kill must occur during the 96 hours.

In the laboratory, place ten middle-sized Daphnia in each of several 4 ounce bottles. Daphnia magna, commonly called water fleas, are small crustaceans. If they cannot be obtained locally, they can be ordered live from a biological supply house. Middle-sized ones are used so that natural mortality due to age, will not lead to incorrect results.

Cover each bottle with nylon cloth having at least 80 threads to the inch. If this kind of cloth is not available, two thicknesses of a piece of nylon stocking will probably be acceptable. The cloth must detain the Daphnia and permit the dissolved substances to diffuse into the bottle. Use rubber bands to fasten the cloth over the opening of the bottle. Place five of these bottles in a 6 inch square wire container (which can be made from 1/4 inch hardware cloth) and place it in the stream. After 96 hours, count and record the number of living adults and offspring.

Small (2 to 3 inch) bluegills (Lepomis macrochirus) and/or largemouth basses (Micropterus salmoides) can also be used. These should be fed and acclimated to laboratory conditions for 2 to 4 days before being placed in the stream.

The fishes can be transported to the bioassay stations in 10-gallon milk containers lined with large plastic bags. If aeration is necessary, use an aquarium pump which can be run off an inverter. The inverter changes 12 volts into 110 volts.

At each station place, 10 bluegills or 10 largemouth basses in a Gee's galvanized, quarter-inch-square wire minnow trap (manufactured by Cuba Specialty Manufacturing Company, Houghton, New York). Close the hole in each end of the minnow trap with a cork or a rubber stopper. This will prevent predators, such as eels, from getting into the trap.

Slowly acclimate the fish to the temperature of the stream. This can be done by putting the fish into a plastic bag (with water from the milk can) and then placing the plastic bag into the stream or else by adding stream water--small amounts at a time--to the plastic bag.

After adding the fish to the trap, place it in 1 or 2 feet of water of very low velocity. If slow-moving water cannot be found, place it on the downstream side of a large rock or other obstruction.

Transfer the living fish from the trap to a plastic bag (after 96 hours). Note the conditions of the surviving fish. Look for vitality or a lack of it. Observe the fins. Some chemical effluents cause them to deteriorate. Record the number of dead fish and the number and conditions of each of the surviving fish.

If these tests are done in highly populated or well used areas you must carefully hide the bottles and traps. If this is not done, they will be disturbed or taken.

Be sure that you do not subject the Daphnia or the fish to temperature shock when placing them in the stream. Daphnia can stand a rapid temperature change of only one to 2° F and the fish can stand a rapid change of only 2° F to 4° F.

f. Diversity Indices

The statistical analysis of some kinds of biological data may be done by computing the diversity index. Generally speaking, the greater the diversity, the healthier the biotic community. There is presently no one method of computing this index, which is universally accepted by the professionals. The methods range from simple ones to others that are best calculated by computers.

The sequential diversity index is explained below. An exercise is included to show the use of this index in the activities section (Chapter 3) of this guide. Others may be found in some of the references listed at the end of this section.

The sequential diversity index is calculated by dividing the number of runs by the number of specimens as show below:

$$\text{Diversity index} = \frac{\text{number of runs}}{\text{number of specimens}}$$

A run is a set of like individuals picked up or observed respectively. A run ends when an individual of another kind is found. Therefore, a run can consist of only one individual. The specimen being observed need only be compared with the previous one. If it appears to be similar, it is part of the same run; if not, it is part of a new run. The more runs for a given number of specimens, the greater the diversity.

Suppose that an investigator is observing cellular algae on a haemocytometer. As he scans the field of view from left to right, he observes the following: 1st, five cells of the same kind which he calls type S; 2nd, ten cells of another kind which he calls type I; 3rd, five cells of type S; and, finally, one cell of type U. At this point, the investigator has four runs and 21 specimens. The usual procedure would be to continue in a like manner until 200 specimens have been counted; then the diversity index would be computed.

Macroinvertebrates may be poured out onto a grid of some sort and treated in the same manner. Alternatively, they may be sorted according to kind. For example, there might have been 80 green, worm-like specimens, 40 snails, and 80 leeches. The green, worm-like specimens would be represented by numbers 1-80, the snails would be 81-120, and the leeches 121-200. Numbered slips of paper would then be randomly drawn to determine the number of runs.

If more than 200 macroinvertebrates are in a sample, randomly pick and sort until 200 specimens have been removed. The remaining organisms can be discarded. (If results from different locations are to be meaningfully compared, the diversity indices should be computed for the same number of specimens.)

5. The Significance and Interpretation of Biological Data

The interpretation of data is a time-consuming process. Biological variability often confuses the beginner. One of the commonest examples of this is that a few individuals of the same species will respond differently than the others to apparently the same environmental conditions. One should be aware of exceptions, but he should not be disturbed if he cannot explain them.

In order to get the most complete picture of an aquatic system, data from as many parameters as possible should be studied. As a matter of fact, data from a single parameter may mean nothing by themselves. Finding interrelationships among the various parameters and relating these to the whole are extremely important.

Care should be exercised to be sure that the data were collected from a representative sample. Following are a series of tables which should aid in the interpretation of data. The information contained in them is not absolute; it should be used only as a guide.

Warning

If the relationships do not seem to apply to your investigation, other factors (perhaps requiring equipment not available to you) may be involved. Rather than risk publicizing an unwarranted conclusion, seek the advice of a professional.

Cross-check related observations in different tables.

For example, if low DO is detected under chemical testing, check turbidity under physical observations and severe organic pollution under biological observations.

The interpretation of phrases such as "great variety," "less variety," and "high coliform count" may pose a problem for beginners. Most states have developed water quality standards which will be helpful in the interpretation of bacterial and some chemical data. If professional macroinvertebrate data (or assistance) are not available to the beginning investigator, he will have to collect extensive data himself; then make his own interpretations.

Table 1 - C - 1 Biological Observations

In Case of:	Look for or Expect:
1. Using Sequential Diversity Index (SDI)	
Great variety with few of each kind	Clean water
Less variety with great abundance	Overly enriched (Moderate organic pollution)
One or two kinds only, with very great abundance	Severe organic pollution

In Case of:	Look for or Expect:
2. The Qualitative Interpretation of Freshwater Macroinvertebrates	
May fly, caddis fly, and stone fly larvae, plus a considerable variety of other macroinvertebrates	Clean water
Pollution tolerant types predominate, although a few less tolerant or unknown forms may be present	Moderate organic pollution Suggestion: Confirm with coliform and other tests.
One or two pollution tolerant types only, often present in overwhelming abundance	Severe organic pollution Suggestion: Same as above.
No macroinvertebrates at all, little or no plant life	Toxic pollution Suggestion: Same as above.
3. Quantitative Interpretation of Freshwater Invertebrates from Riffle Areas	
Note: Carefully review weather records for the preceding few weeks. A severe flood could invalidate the following interpretations.	
0 - 2 grams per ft.^{2} (blotted live weight)	Unproductive, probably clean stream. Suggestion: Check for toxicity.
3 - 5 grams per ft.^{2}	Normally productive. Probably well balanced stream community.
Over 6 grams per ft.^{2}	A. Highly productive stream, probably organically enriched (polluted).

In Case of:	Look for or Expect:
	B. Note: These relative values will differ in different parts of the country. C. Check the SDI.
4. Productivity Measurements	
Water at sampling site seems to be unproductive or overproductive	Suggestion: Measure plankton productivity using standing crop, oxygen, pH, or carbon-14 method (or combination).
5. Fish Behavior	
Many fish observed to be "topping" (gulping air and/or splashing on surface)	Suggestion: 1. Check DO, IDOD, and BOD. 2. Check for toxic or oxygen demanding chemicals. 3. Determine organic content of water and bottom debris and/or sediments. 4. Check temperature.

Table 1 - C - 2 Bacteriological Observations

In Case of:	Look for or Expect:
High coliform count	A. Raw or unchlorinated sewage discharge. B. Pasture or feed lot drainage.

In Case of:		Look for or Expect:
	C.	Storm sewer drainage immediately after a rain storm.
Low coliform count	A.	Clean water.
	B.	Heavily chlorinated sewage effluent.
	C.	Toxic discharge as from pharmaceutical company manufacturing antibiotics, or toxic chemicals.
	D.	Other source of toxicity such as acid mine drainage.

Table 1 - C - 3 Chemical Observations

In Case of:		Look for or Expect:
High DO's (12-30 mg/l) during daylight hours (supersaturation)	A.	High biological productivity, especially producers (plants).
	B.	Relatively quiet waters.
	C.	Chemical interference in oxygen determination.
	Suggestion:	
	1.	Check DO between 2 and 3 a.m.
	2.	Check DO at 1 or 2 hour intervals around the clock and graph results.
	3.	Search for source of excessive fertility.
	4.	Examine bottom muds for black anaerobic foul smelling (H_2S) deposits that are overgown by plants.

In Case of	Look for or Expect:
	5. Compare DO above and below a dam or rapids area. Deaeration may be detected.
Low DO's (0-4 mg/l) during daylight hours	A. High organic content, both dissolved and suspended solids. B. High total bacteria and fungus count. C. If clear water, look for anaerobic spring (groundwater). D. Chemical interference in oxygen determination. E. Note water temperature. Suggestion: 1. Check for coliform bacteria. 2. See "Physical, Low Velocity" (Suggestion #1).
Toxic chemicals in general	Reduced biological productivity may be selective or complete.
Toxic or smothering chemicals which sink to bottom	No living organisms on or in bottom materials, but overlying water may have rich plankton and/or nekton population.
Floating oil slick	A. Low DO near surface. B. Oil coated wharf pilings, floats, and shore. C. Dead or dying oil soaked birds and aquatic mammals. D. Few or no living organisms on oil covered surfaces. If in an estuary or open ocean front shore, this includes the entire intertidal zone.

In Case of:	Look for or Expect:
High pH (8-11), high alkalinity (200 or more), high hardness (300 or more)	A. High turbidity.
	B. High biological productivity.
	C. If low biological production, look for toxicity or biologically intolerable combination of chemicals.
	D. May be of natural origin.
Low pH (2-5)	A. Acid mine drainage or industrial discharge.
	B. Low biological productivity.
	C. Low turbidity.
Specific chemical effects	See Table 1-D of Appendix I

Table 1 - C - 4 Physical Observations

In Case of:	Look for or Expect:
High velocity and turbulence	A. DO approximately at saturation for temperature.
	B. Hard bottom, little sediment.
	C. Biological organisms adapted to swift water.
	D. Particulate materials kept in suspension.
	E. Bank and bottom scouring (erosion).

In Case of:	Look for or Expect:
Low velocity	A. DO may be above or below saturation.
	B. Coarse particulates may settle to bottom.
	C. Bottom may be soft.
	D. Organisms, if present, may be burrowers or may crawl freely on surface.
	Suggestions:
	1. If turbidity is high, see High Velocity, Item D.
	2. Check for kinds and amounts of plankton.
High volume of flow: normal, non-flood conditions (over 1,000 c.f.s.)	A. Note: This is probably a "big" river.
	B. These are difficult and expensive to study, even for professional groups.
	Suggestions:
	1. Examine the water for plankton.
	2. Place artificial substrates for both periphyton and macroinvertebrates.
	3. Carry out chemical and physical analyses of water.
Flood conditions (any stream)	A. High coliforms count in first few hours, diminishing as time goes on.
	B. "Dumping" of waste holding ponds by industry.
	C. "By-passing" of sewage treatment plants.

In Case of:	Look for or Expect:
	D. See also "physical" High Velocity, Item D, and Low Velocity, Item B.
Low volume of flow (up to 1,000 c.f.s.)	A. Note: Smaller streams from 0 up to 200 - 300 c.f.s. are generally most satisfactory for group studies but much depends on local circumstances, resources, and objectives.
	B. Most of the analyses described can be carried out on such a stream.
Heated water discharge (no chemical pollution assumed although heated discharges often contain chlorine or other chemicals used to kill biological growths in the plant. In this case, see also "Toxic Chemical" section.)	A. Differences between the biota in or near the discharge canal or pipe and that in or around the intake.
	B. Artificial substrates may be used.
	Suggestions:
	1. Make the above comparison winter and summer, or even better, each season.
	2. Chart the dispersal of the heated water on the receiving water at different times of the year, different wind directions, different tidal phases, etc. If available, use depth recording thermometer and include depth as well as surface temperature. Graph your results.
Lakes, reservoirs, and estuaries	A. Thermal (or other density caused) stratification.
	B. Changes in water level, either natural or man made.
	Suggestions:
	1. Practically every suggestion offered elsewhere for stream or

In Case of:	Look for or Expect:
	river pollution studies can be applied to lakes, reservoirs, or estuaries, making due allowance for differences in the basic nature of the waters.
	2. Stratification, seiches, density currents, tidal currents, and salinity are additional physical factors to be considered.
	3. Biological procedures are virtually identical, but while DO is the same, most of the chemical methods cited apply to freshwater only.

Appendix 1

Figure 1 - C - 1 Benthos Responses to Pollution

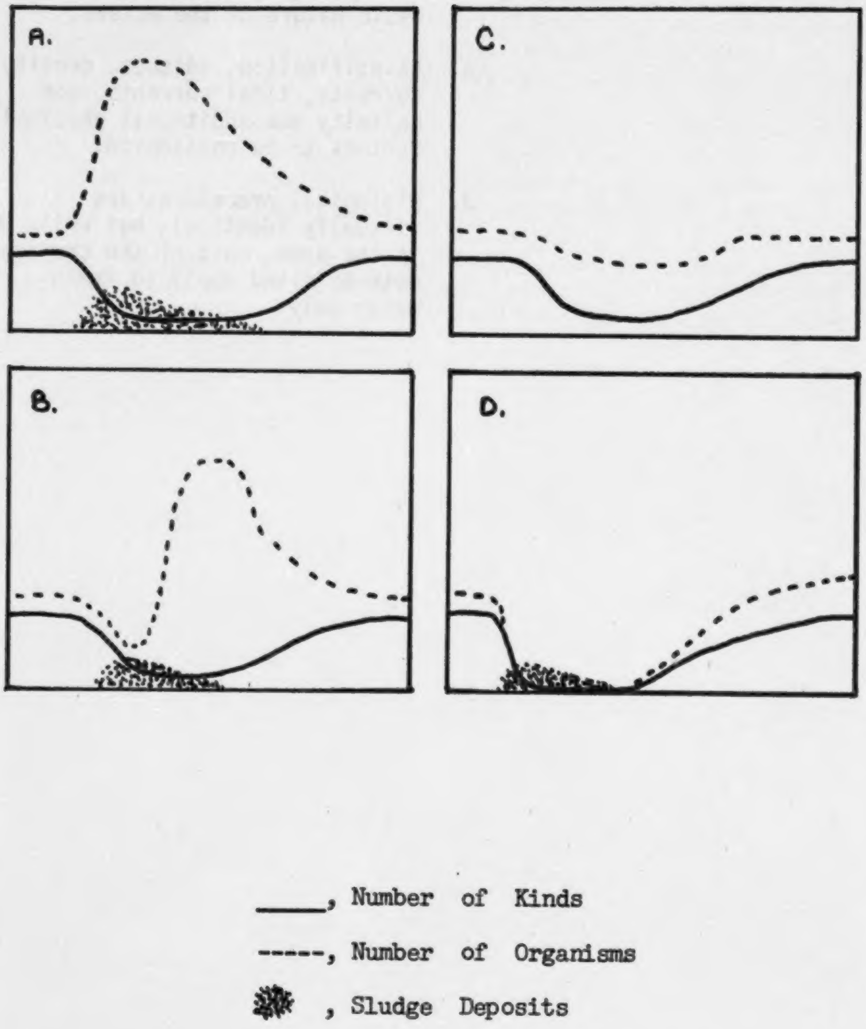

A.
C.
B.
D.
____, Number of Kinds
-----, Number of Organisms
, Sludge Deposits

Figure 1 - C - 1

Four Basic Responses of Bottom Animals to Pollution

A. Organic wastes eliminate the sensitive bottom animals and provide food in the form of sludges for the surviving tolerant forms.

B. Large quantities of decomposing organic wastes eliminate sensitive bottom animals and the excessive quantities of by-products of organic decomposition inhibit the tolerant forms; in time, with natural stream purification, water quality improves so that the tolerant forms can flourish, utilizing the sludges as food.

C. Toxic materials eliminate the sensitive bottom animals; sludge is absent and food is restricted to that naturally occurring in the stream, which limits the number of tolerant surviving forms. Very toxic materials may eliminate all organisms below a waste source.

D. Organic sludges with toxic materials reduce the number of kinds by eliminating sensitive forms. Tolerant survivors do not utilize the organic sludges because the toxicity restricts their growth.

6. Bibliography

Cairns, J., Jr., et al, "The Sequential Comparison Index--A Simplified Method for Nonbiologists to Estimate Relative Differences in Biological Diversity in Stream Pollution Studies," Journal, Water Pollution Control Federation, 1968, pp. 1607-1613. This is an excellent article and is highly recommended.

Clark, W. J., and W. F. Sigler, "Method for Concentrating Phytoplankton Samples Using Membrane Filters," Journal of Limnology and Oceanography, 1963, 8 (1): 127-129.

Eddy, S., How to Know the Fresh-Water Fishes, Wm. C. Brown Co., Dubuque, Ia., 1957. It is a fairly good reference which can easily be carried into the field.

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Gardiner, A. C., "Measurement of Phytoplankton Population by the Pigment Extraction Method," Journal of the Marine Biological Association, 1943, 25 (4): 739-744.

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A List of Common and Scientific Names of Fishes from the United States and Canada, American Fisheries Society, Publication #2, Washington, D. C., 1960.

Pennak, R. W., Fresh-Water Invertebrates of the United States, Ronald Press, New York City, 1953. This is a good general reference but very technical.

Pollison, D. P., and W. M. Craighead, Lehigh River Biological Investigation, Delaware River Basin Commission, Trenton, N. J., 1968, pp. 24-25. This is a comprehensive report on a river study. A biomonitoring technique is described; the report calls it a bioassay.

Needham, J. G., and P. R. Needham, A Guide to the Study of Fresh-Water Biology, Holden-Day, Inc., San Francisco, Calif., 1962. It is a good reference book with excellent drawings of organisms.

Sources of Limnological and Oceanographic Apparatus and Supplies, American Society of Limnology and Oceanography, Publication #2, Washington, D. C., 1961. Many specialized items of biological collecting equipment are not available from the usual supply houses. This publication lists the suppliers.

Standard Methods for the Examination of Water and Wastewater, (13th ed.), American Public Health Association, Inc., New York City, 1971. This book discusses the physical, chemical, and biological analytical techniques, bioassays, and chemical analysis. It has good drawings and keys of organisms. Every school should have at least one copy.

Wilhm, J. L., "Patterns of Numerical Abundance of Animal Populations," The American Biology Teacher, March 1969, pp. 147-150. A diversity index is presented as well as other means of statistically analyzing biological data.

Wilhm, J. L., and T. C. Dorris, "Biological Parameters for Water Quality Criteria," Biological Science, June 1968, 18-6: 477-481. A comprehensive discussion of the diversity index is given.

D. Engineering and Physics

A number of useful tools which relate to the previously discussed chemical, bacteriological, and biological parameters are used in water studies. In the Engineering and Physics Section, mapping, flow measurements and computer use are discussed as they apply to water pollution and water surveys.

Map reading can facilitate water survey planning and provide a better understanding of the body of water being studied. Flow measurement provides valuable data in interpreting variations in results from chemical, bacteriological, and biological samplings. The computer is a valuable tool in compiling sampling data. A working knowledge in these three areas will aid in water studies and the interpretation of collected data.

1. Mapping

In water surveys, maps provide an invaluable tool for the recording of sampling sites and for providing information on water sources and posssible points of pollution. For example, in the initial planning of a river study a topgraphic map is useful for indicating incoming streams and whether those streams pass through populated areas. Local sewage pipe line maps, obtained through the city engineer or department of public works, will pinpoint sources. Thus, before any field work is done, a fairly good idea of possible sources of pollution may be obtained through the use of maps.

When a small area is being studied, such as a pond, a map can be easily drawn using the plane-table survey method. This method may be used in grade levels 6-12.

Plane-Table Survey Method

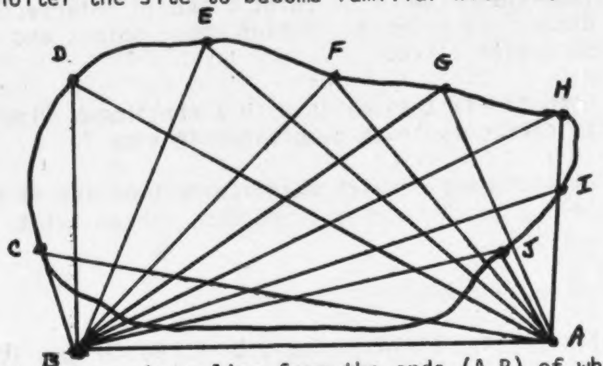
a. Equipment

1) Tape measure
2) Wooden stakes
3) A light weight table or head board with some type of support (i.e., legs)
4) Paper, pencil, ruler
5) Plumb line or carpenter's level

b. Procedure

Although the following procedure is given for mapping a small pond, the same procedure may be used to map a parking lot, a school playground, a woodlot, etc. In mapping areas such as these, sightings would be taken to existing objects (i.e., trees, parked cars, swings) rather than driving stakes.

Before the following procedure is started, the mappers should reconnoiter the site to become familiar with it.



1) Determine a base line from the ends (A,B) of which almost all points on the shore line (C-J) are visible. Place stakes at point A and B.

2) Place stakes along the shoreline (C-J) so that they are visible from points A and B.

3) Place the table or head board at point A. Using the plumb line or carpenter's level, make sure the table is horizontal.

4) Tape a piece of paper to the table. At eye level to the table, line up stake B with a ruler and draw a line toward the sighting. This line is called the base line.

5) From point A line up the other stakes (C-J) with a ruler and draw a line along the line of sight.

6) Measure the distance between point A and point B with a tape measure.

7) On the base line sketched in step 4) place point B according to the scale desired. For instance, if the distance between point A and B is 100 feet, point B could be placed 10 inches from point A on the sketched base line. This would give a scale of "1 inch equals 10 feet."

8) Move the table to point B and again make sure the table is horizontal.

9) Align the map so that point A may be seen by placing a ruler along the base line and sighting along the top of the ruler.

10) As soon as the base line is aligned, sight points C-J and draw the lines toward the sightings.

11) The lines drawn from point B should intersect those lines drawn from point A. Darken those points and erase the construction lines.

12) Connect all the points with a continuous line. The map is now to scale as determined in step 7.

13) Fill the map in with whatever information is pertinent (e.g., north-bearing direction, stream inlets, houses, etc.).

c. References and Resources

Phillips, E.A., Field Ecology, D. C. Heath Co., Boston, 1964.

Welch, P. S., Limnological Methods, McGraw-Hill Book Co., New York City, 1948.

Topographic maps of any area in the United States may be obtained through the Geological Survey, Department of the Interior, Distribution Section, Washington, D. C. State index circulars and a folder describing topographic maps may be obtained free from the above address.

2. Flow

Flow measurements, the velocity and volume of water in a stream, are among the more important data collected in a water survey.

The velocity of water movement will determine the types of organisms living in a particular segment of a stream. Likewise, the velocity will affect the transport of nutrients and organic food past those organisms attached to stationary surfaces; the transport of plankton and benthos as drift, which in turn serve as food for higher organisms; the transport of silts and sediments; and the addition of dissolved oxygen through surface aeration.

The volume of water in a stream determines to what extent toxic substances and bacteria are diluted and, therefore, the immediate effects of an effluent on a stream's condition. Flow measurements should be determined whenever chemical, bacteriological, or biological samplings are taken. Variations in chemical, bacteriological, or biological results can often be attributed to flow variations.

Appendix 1

Elaborate apparatus is usually used in flow gauging studies, such as current meters and weirs. The floatation method of estimating flow, however, can be achieved with simple equipment by students in the 7th - 12th grades.

a. Velocity

1) Equipment

a) A floating object (float) (This float is to be carried along by the water and should be as immune as possible to air flow and should be as visible as possible. In deep water, an orange will satisfy the needs. For smaller streams, smaller floats such as corks or rubber balls have been found very useful.)

b) Measuring tape or calibrated rope in feet

c) Stopwatch or watch with second hand

d) Boots

2) Procedure

a) It is most difficult to measure flow in slowly moving waters that are over 4 feet deep. This would mean that a stream with rapids would be best to use for the float.

b) Locate two points (parallel to flow) in center of stream, any measurable distance apart.

c) Measure and record the distance between the two points, making sure that the area is free of any obstructions (rocks, garbage, etc.).

d) The moment you place the float at the upstream point, start timing.

e) Mark the time at the instance the float passes the downstream point.

f) Using the formula, $V(\text{Velocity}) = \frac{D(\text{Distance})}{T\ (\text{Time})}$, compute the value.

Note: Average velocity should be calculated to determine the true velocity of the stream. It can be concluded that the water of a stream or river will flow fastest on the surface at the center, and that the average velocity of a stream will, therefore, be less than the surface velocity.

g) To obtain the average velocity, multiply the surface velocity by the constant bottom type factor, 0.9 for smooth bottomed streams or rivers (sand, clay, etc.) and 0.8 for rough bottomed streams or rivers (rocks, debris, etc.).

b. Volume

1) Equipment

a) A meter, yard stick or measuring tape for measuring the depth and width

b) A slide rule, if desired, for calculating the results

2) Procedure

a) Find the average width in feet of the stream between the same two points used in velocity measurements by finding the width at regular intervals between the points and taking the average of the widths.

b) Determine the average depth in feet of the streams between the points, a certain number of depths from one side of the stream to the other. Take the average of these depths at each interval, and then take the average of the average depths for the entire distance between the two points.

c) Compute the average cross section of the stream in square feet between the two points by multiplying the average width by the average depth.

d) Compute the volume of flow in second-feet (sec./ft.) by multiplying the average cross section by the average velocity in feet per second, obtained from the velocity calculations. The breakdown of units of measurement in the calculations is as follows:

$$(\text{feet})^2 \times \frac{\text{feet}}{\text{sec.}} = \frac{(\text{feet})^3}{\text{sec.}} \text{ or ft.}^3/\text{sec.}$$

When flow measurements are being taken from a stream or river in which wading would be dangerous or impossible, a boat may be used or measurements may be taken from a bridge.

c. References

Mackenthun, Kenneth M., The Practice of Water Pollution Biology, U. S. Department of the Interior, Washington, D. C., 1969. This paperback contains only half a page on flow but it is a good text for aquatic biology.

Appendix 1

Manual on Water, (3rd ed.), American Society for Testing and Materials, Philadelphia, Pa., 1969. This text has a chapter on flow measurement but deals with the equipment of interest to industries.

Grover, N. C., and Harrington, A. R., Stream Flow, Dover Publications, New York City, 1966. This is a good paperback dealing, among other things, with methods and instruments for measuring stream flow.

E. Computer Applications

The volumes of data generated from the activities in this text can be handled most efficiently by a digital computer. The computer's speed and accuracy is readily adapted to the field of pollution studies as a tool for the ecologist. Though not everyone is an accomplished programmer, programs can be generated in the timeshare "BASIC" language that can be run by almost everyone.

The following programs are written with the nonprogrammer in mind. By using the Dartmouth College computer, through a remote teletype terminal, anyone who can type can access the system, call up the appropriate program (title known from an annotated catalog), and run that program to obtain the necessary results from his experiment. The conversational nature of the Dartmouth "BASIC"[1] langugage permits the programmer to write a "prompting" type of program, so that the user gets the feeling that he is having a "conversation."

These programs are not restricted to the Dartmouth system alone. With minor changes, they can be accepted by most timeshare systems, or can be rewritten in Fortran for nontimeshare systems (although they will lose their conversational nature). Local conditions will dictate how the computer can be adapted as a tool for the ecologist.

1. STREAM

STREAM calculates the cross section, velocity and volume of flow of a stream. Input the site information (site number and location), the distance in feet from one depth reading to the next, the depth at each reading in feet and inches, the number of velocity trials, the distance in feet and inches of each trial, and the travel time in seconds for each trial. The method of calculating cross section uses a series of triangular and rectangular areas, where field accuracy dictates volumetric accuracy. The output is a summary of cross sectional area in $ft.^2$, stream velocity in ft./sec. and volume of flow in $ft.^3$/sec. A question is then asked as to whether or not you want a plot. A "yes" will produce a plot of the cross section of the stream at the site of study. A "RUN" and "LIST" follow.

---

[1] BASIC Programming,(Preliminary 5th ed.), Kemeny and Kurtz, Dartmouth Press, Hanover, N. H., 1969.

```
RUN

STREAM    09 AUG 70  20:39


THIS PROGRAM CALCULATES CROSSECTION,VELOCITY,FLOW VOLUME
AND PLOTS THE CROSSECTIONAL PROFILE.

DIRECTIONS: ANSWER COMPUTER QUESTIONS.

                STREAM CROSSECTION CALCULATION.

SITE NO.? 3
LOCATION? WINNESQUAM RIVER
WIDTH OF STREAM (F,I)? 50,0
HOW MANY DEPTH READINGS WERE TAKEN? 13
DISTANCE FROM SHORE TO FIRST MEASUREMENT(FT.) AND DEPTH (F,I)? 5,2,0
DISTANCE(F),DEPTH(F,I)? 3,3,0
DISTANCE(F),DEPTH(F,I)? 2,4,0
DISTANCE(F),DEPTH(F,I)? 2,4,0
DISTANCE(F),DEPTH(F,I)? 3,5,0
DISTANCE(F),DEPTH(F,I)? 2,6,0
DISTANCE(F),DEPTH(F,I)? 3,7,0
DISTANCE(F),DEPTH(F,I)? 4,5,0
DISTANCE(F),DEPTH(F,I)? 3,4,0
DISTANCE(F),DEPTH(F,I)? 3,3,0
DISTANCE(F),DEPTH(F,I)? 5,2,0
DISTANCE(F),DEPTH(F,I)? 5,1,0
DISTANCE(F),DEPTH(F,I)? 5,1,0
DISTANCE FROM LAST DEPTH TO SHORE? 5

                AVERAGE VELOCITY CALCULATION.

HOW MANY TRIALS WERE CONDUCTED? 2
DISTANCE BETWEEN POINTS(FT.,IN.)? 48,0
TIME OF FLOAT(SEC.)? 16.2
DISTANCE BETWEEN POINTS(FT.,IN.)? 50,0
TIME OF FLOAT(SEC.)? 17.5
WAS THE STREAM 1)SMOOTH OR 2) ROUGH BOTTOMED? 1

DATA FOR:WINNESQUAM RIVER

THE AVERAGE VELOCITY OF STREAM AT SITE 3  IS  2.62 FT./SEC.

THE CROSSECTION IS  147  SQ.FT.

THE VOLUME OF FLOW IS  385.1  FT.↑3/SEC.

 DO YOU WISH A PLOT? YES
```

```
          CROSSECTION AT SITE  3  ON WINNESQUAM RIVER

MEASUREMENT                  WATER LEVEL

BANK                              *
                                  .
                                  .
                                  .
                                  .
 5   ( 2 )                      * .
                                  .
                                  .
 8   ( 3 )                     *  .
                                  .
 10  ( 4 )                    *   .
                                  .
 12  ( 4 )                    *   .
                                  .
                                  .
 15  ( 5 )                   *    .
                                  .
 17  ( 6 )                  *     .
                                  .
                                  .
 20  ( 7 )                 *      .
                                  .
                                  .
                                  .
 24  ( 5 )                   *    .
                                  .
                                  .
 27  ( 4 )                    *   .
                                  .
                                  .
 30  ( 3 )                     *  .
                                  .
                                  .
                                  .
                                  .
 35  ( 2 )                      * .
                                  .
                                  .
                                  .
                                  .
 40  ( 1 )                       *.
                                  .
                                  .
                                  .
                                  .
 45  ( 1 )                       *.
                                  .
                                  .
                                  .
                                  .
BANK ( 50 )                       *

DO YOU HAVE ANOTHER SITE TO CALCULATE? NO
```

STREAM

```
100'
110' CALCULATES CROSSECTION,VELOCITY,FLOW VOLUME AND PLOTS SITE PROFILE.
120'
130 PRINT
140 PRINT
150 PRINT "THIS PROGRAM CALCULATES CROSSECTION,VELOCITY,FLOW VOLUME "
155 PRINT "AND PLOTS THE CROSSECTIONAL PROFILE."
170 PRINT
180 PRINT "DIRECTIONS: ANSWER COMPUTER QUESTIONS."
190 PRINT
200 PRINT TAB(15);"STREAM CROSSECTION CALCULATION."
210 PRINT
220 PRINT "SITE NO.";
230 INPUT S1
240 PRINT "LOCATION";
250 INPUT L$
260 PRINT "WIDTH OF STREAM (F,I)";
270 INPUT W,W5
280 LET W=W+W5/12
290 PRINT "HOW MANY DEPTH READINGS WERE TAKEN";
300 INPUT R
310 PRINT"DISTANCE FROM SHORE TO FIRST MEASUREMENT(FT.) AND";
320 PRINT " DEPTH (F,I)";
330 INPUT D(1),H(1),I(1)
340 LET H(1)=H(1)+I(1)/12
350 LET W1=D(1)
360 FOR J=2 TO R
370 PRINT "DISTANCE(F),DEPTH(F,I)";
380 DIM D(20),H(20), I(20)
390 INPUT D(J),H(J),I(J)
400 LET H(J)=H(J)+I(J)/12
410 LET W1=W1+D(J)
420 NEXT J
430 PRINT "DISTANCE FROM LAST DEPTH TO SHORE";
440 INPUT D(R+1)
450 LET W1=W1+D(R+1)
460 IF W1=W THEN 540
470 PRINT
480 PRINT "ERROR IN WIDTH MEASUREMENT."
490 PRINT
500 PRINT "DO YOU WISH TO CONTINUE";
510 INPUT R$
520 IF R$="YES" THEN 540
530 STOP
540 LET T=.5*D(1)*H(1)
550 LET W=W1
560 FOR I=1 TO R-1
570 IF H(I+1)>H(I) THEN 610
580 LET T=T+.5*(H(I)-H(I+1))*D(I+1)+D(I+1)*H(I+1)
590 NEXT I
```

STREAM (CONTINUED)

```
600 GO TO 630
610 LET T=T+.5*(H(I+1)-H(I))*D(I+1)+H(I)*D(I+1)
620 GO TO 590
630 LET T=T+.5*D(R+1)*H(R)
640'VELOCITY
650 PRINT
660 PRINT TAB(15);"AVERAGE VELOCITY CALCULATION."
670 PRINT
680 PRINT "HOW MANY TRIALS WERE CONDUCTED";
690 INPUT K
700 LET V=0
710 FOR J=1 TO K
720 PRINT "DISTANCE BETWEEN POINTS(FT.,IN.)";
730 INPUT F(J),I(J)
740 LET F(J)=F(J)+I(J)/12
750 PRINT "TIME OF FLOAT(SEC.)";
760 INPUT S(J)
770 LET V=F(J)/S(J)+V
780 NEXT J
790 LET V=V/K
800 PRINT "WAS THE STREAM 1)SMOOTH OR 2) ROUGH BOTTOMED";
810 INPUT A
820 IF A=2 THEN 850
830 LET V=V*.9
840 GO TO 860
850 LET V=V*.8
860 PRINT
870 PRINT "DATA FOR:";L$
880 PRINT
885 LET V=INT(100*V+.5)/100
890 PRINT "THE AVERAGE VELOCITY OF STREAM AT SITE";S1;" IS ";
900 PRINT V;"FT./SEC."
910 PRINT
915 LET T=INT(100*T+.5)/100
 20 PRINT "THE CROSSECTION IS ";T;" SQ.FT."
930 PRINT
935 LET F=INT(10*T*V+.5)/10
940 PRINT "THE VOLUME OF FLOW IS ";F;" FT.↑3/SEC."
950 PRINT
960 PRINT " DO YOU WISH A PLOT";
970 INPUT R$
980 IF R$="YES" THEN 1000
990 STOP
1000 GOSUB 1060
1010 PRINT
1020 PRINT"DO YOU HAVE ANOTHER SITE TO CALCULATE";
1030 INPUT R$
1040 IF R$="YES" THEN 190
1050 STOP
1060'PLOT
```

STREAM (CONTINUED)

```
1070 PRINT
1080 PRINT
1090 PRINT
1100 PRINT TAB(10);"CROSSECTION AT SITE ";S1;" ON ";L$
1110 PRINT
1120 PRINT "MEASUREMENT";TAB(29);"WATER LEVEL"
1130 PRINT
1140 PRINT "BANK";TAB(34);"*"
1150 LET J=1
1160 LET D9=D(J)
1170 FOR X=1 TO W-1
1180 IF X=D9 THEN 1210
1190 PRINT TAB(34);"."
1200 GO TO 1250
1210 IF H(J)<1 THEN 1270
1220 PRINT X;TAB(5);"(";H(J);")";TAB(34-INT(H(J)+.5));"*";TAB(34);"."
1230 LET J=J+1
1240 LET D9=D9+D(J)
1250 NEXT X
1260 GO TO 1290
1270 PRINT X;TAB(33);"*."
1280 GO TO 1230
1290 PRINT "BANK (";W;")";TAB(34);"*"
1300 RETURN
1310 END
```

2. DIV

DIV calculates the diversity index of a sample in a biotic community using the information and formulas presented in the article "Biological Parameters for Water Quality Criteria" by Jerry L. Wilhm and Troy C. Dorris presented in Bioscience, Vol. 18, No. 6. The diversity index is an indication of pollution levels, for "values less than 1 have been obtained in areas of heavy pollution, values from 1 to 3 in areas of moderate pollution, and values exceeding 3 in clean water areas."[1] Refer to Activity E, Chapter 3.

This program is not conversational in nature. Data must be inserted as "DATA" statements beginning with line 720. These "DATA" statements must be of the following form: 146, 5, 131, 7, 4, 1, 3, 1, 1.86, etc., where 146 is the total number of individuals, 5 the number of individual types, 131 through 3 the numbers per individual population, 1 the site number, and 1.86 the biomass of that sample.

The output is a table listing the individual diversity, diversity index, theoretical maximum and minimum diversity, and a redundancy factor. Redundancy expresses the dominance of a type, while diversity shows the compositional richness of a mixed population aggregation of organisms.

A "RUN" and "LIST" follows.

---

[1] "Biological Parameters for Water Quality Criteria," Wilhm and Dorris, Bioscience, Vol. 18, No. 6, 1968.

```
RUN

DIV       09 AUG 70  19:50

          INDIVIDUAL                      MAXIMUM    MINIMUM
LOCATION  DIVERSITY  DIVERSITY  DIVERSITY  DIVERSITY  BIOMASS    R


      1    3.94323    0.657052   2.26021    0.196572   1.86  0.776861

      2    4.15551    1.34916    2.27043    0.165477   1.05  0.437667

      3    4.3029     0.777571   2.44827    0.184772   1.5   0.738105

      4    3.74413    0.49188    1.87177    0.173511   0.2   0.812532

      5    4.96296    1.01232    2.51868    0.107735   0   0.624798

      6    5.38642    5.36488 E-2   1.97682  4.63947 E-2   6.1   0.996242

      9    4.2596     1.59888    2.94719    0.30109    2.3   0.509546

     10    3.07844    1.27176    2.25769    0.465142   0.15   0.550017

     11    3.93598    0.583135   2.19182    0.198744   0.64   0.807137

     12    3.22023    1.01045    2.38556    0.425624   0.67   0.70161

     13    4.79031    0.730937   1.94339    7.50793 E-2  0.91   0.648957

     14    2.79423    1.11113    1.82516    0.347148   2.36   0.483105

     15    4.92       0.711892   2.50029    0.112172   5.48   0.748873

     16    3.15433    0.820883   1.86566    0.267617   0.85   0.653786

     17    5.21978    0.259188   2.73366    0.105908   5.4   0.941669

     18    2.05037    1.80127    1.8543     0.748345   1.8   0.04795

     19    5.24932    0.20262    2.55626    8.62842 E-2   6.01   0.9529

     20    3.46477    1.45072    2.3364     0.346989   1.5   0.445195

     21    4.53496    0.830485   2.49195    0.152622   1.8   0.710232

     22    3.31442    1.62428    2.46072    0.466243   1.5   0.419378
```

R IS A REDUNANCY EXPRESSION WHICH IS THE
DOMINANCE OF ONE OR MORE SPECIES AND IS THE
INVERSE PROPORTION TO THE WEALTH OF THE SPECIES.

```
DIV

100 REM    SOURCE:BIOLOGICAL PARAMETERS FOR WATER QUALITY CRITERIA
110 REM    BY WILHM AND DORRIS   FROM BIOSCIENCE VOL.18 NO.6
120 REM
130 REM    PREPARED BY WPP - TILTON   SUMMER '70
140 REM
150 PRINT TAB(10);"INDIVIDUAL";TAB(32);"MAXIMUM";TAB(43);"MINIMUM"
160 PRINT "LOCATION";TAB(10);"DIVERSITY";TAB(21);"DIVERSITY";TAB(32);
170 PRINT "DIVERSITY";TAB(43);"DIVERSITY";TAB(54);"BIOMASS    R"
180 PRINT
190 PRINT
200 PRINT
210 GOSUB 310
220 GOSUB 460
230 GOSUB 530
240 GOSUB 620
250 READ O,R
260 IF O<10 THEN 700
270 PRINT TAB(2);O;
280 PRINT TAB(10);Q;TAB(21);E;TAB(32);M(1);TAB(43);
290 PRINT M(2);TAB(54);R;(M(1) -E)/(M(1)-M(2))
300 GOTO 200
310 DIM N(72),A(50)
320 READ Z
330 IF Z=0 THEN 840
340 LET N=LOG(Z)
350 FOR T=Z-1 TO 1 STEP -1
360 LET N=LOG(Z-T)+N
370 NEXT T
380 READ S
390 LET B=0
400 FOR C=1 TO S
410 READ A (C)
420 LET B=LOG(A(C))+B
430 NEXT C
440 LET Q=(1/Z)*(N-B)
450 RETURN
460 DIM E(72)
470 LET D=0
480 FOR I=1 TO S
490 LET D=(A(I)/Z)*LOG(A(I)/Z)/LOG(2)+D
500 NEXT I
510 LET E=-D
520 RETURN
530 LET F=N/LOG(2)
540 LET G=INT(Z/S+.5)
550 LET H=LOG(G)
560 FOR C=G-1 TO 1 STEP -1
570 LET H=LOG (C)+H
580 NEXT C
590 LET G=H/LOG(2)
```

DIV (CONTINUED)

```
600 LET M(1)=(F-S*G)/Z
610 RETURN
620 LET J=Z-(S-1)
630 LET K=LOG(J)
640 FOR C=J-1 TO 1 STEP -1
650 LET K=LOG(C)+K
660 NEXT C
670 LET K=K/LOG(2)
680 LET M(2)=(F-K)/Z
690 RETURN
700 PRINT TAB(3);O;
710 GO TO 280
720 DATA 146,5,131,7,4,1,3,1,1.86,181,5,99,68,1,1,12,2,1.05,208
730 DATA 6,180,1,18,3,1,5,3,1.5,119,4,110,4,1,4,4,.2,401,6,326
740 DATA 17,9,1,6,42,5,0,596,4,593,1,1,1,6,6.1,203,9,118,2,61
750 DATA 11,2,1,1,6,1,9,2.3,64,6,48,2,9,2,2,1,10,.15,144,5,131
760 DATA 6,5,1,1,11,.64,72,6,59,7,1,1
770 DATA 3,1,12,.67,335,4,288,15,31,1,13,.91,48,4,35,1,2,10
780 DATA 14,2.36,382,6,336,5,30,1,8,2,15,5.48,68,4,58,2,3,5
790 DATA 16,0.85,509,7,494,3,2,1,2,6,1,17,5.40,24,5,8,1,3
800 DATA 1,11,18,1.8
810 DATA 523,6,511,1,6,1,2,2,19,6.01,94,6,62,3,21,1,2,5,20,1.5
820 DATA 263,6,228,9,13,9,3,1,21,1.8,81,7,3,2,30,41,1,2,1,22,1.5
830 DATA 0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0
840 PRINT
850 PRINT
860 PRINT
870 PRINT
880 PRINT
890 PRINT
900 PRINT "R IS A REDUNANCY EXPRESSION WHICH IS THE"
910 PRINT "DOMINANCE OF ONE OR MORE SPECIES AND IS THE"
920 PRINT "INVERSE PROPORTION TO THE WEALTH OF THE SPECIES."
930 END
```

3. DIVERS

DIVERS is similar to DIV, except it incorporates Stirling's formula, $H = {}^{-}E\ (Ni/N) \log_2 (Ni/N)$[1],to indicate population diversity where N indicates total population count and Ni indicates count per individual type. The run time of this program is far less than DIV, and the printout also gives a comment as to pollution level as noted under the comment for DIV.

This program is also nonconversational in nature and you must replace the data using "DATA" statements after line 370. The form is as follows: 1, 146, 5, 131, 7, 4, 1, 3, etc. Where 1 is the site number, 146 the total population, 5 the number of individual types, and 131-3 the number of each individual type.

A "RUN" and "LIST" follows.

---

[1] "Biological Parameters for Water Quality Criteria," Wilhm and Dorris, Bioscience, Vol. 18, No. 6, 1968.

```
RUN

DIVERS     09 AUG 70  19:57

LOCATION  DIVERSITY           COMMENT


   1        0.657052       HEAVY POLLUTION.
   2        1.34916        MODERATE POLLUTION.
   3        0.777571       HEAVY POLLUTION.
   4        0.49188        HEAVY POLLUTION.
   5        1.01232        MODERATE POLLUTION.
   6        5.36488 E-2    HEAVY POLLUTION.
   9        1.59888        MODERATE POLLUTION.
   10       1.27176        MODERATE POLLUTION.
   11       0.583135       HEAVY POLLUTION.
   12       1.01045        MODERATE POLLUTION.
   13       0.730937       HEAVY POLLUTION.
   14       1.11113        MODERATE POLLUTION.
   15       0.709969       HEAVY POLLUTION.
   16       0.820883       HEAVY POLLUTION.
   17       0.259188       HEAVY POLLUTION.
   18       1.80127        MODERATE POLLUTION.
   19       0.20262        HEAVY POLLUTION.
   20       1.45072        MODERATE POLLUTION.
   21       0.830485       HEAVY POLLUTION.
   22       1.62666        MODERATE POLLUTION.

TIME: 0.168 SEC.
READY
```

DIVERS

```
100'
110'  SOURCE:BIOLOGICAL PARAMETERS FOR WATER QUALITY CRITERIA
120'  WILHM AND DORRIS - BIOSCIENCE VOL. 18 NO. 6
130'
140'  DIVERSITY USING STIRLING'S FORMULA
150'
160 PRINT
170 PRINT "LOCATION"; TAB(10); "DIVERSITY"; TAB(30); "COMMENT"
180 PRINT
190 PRINT
200 READ L,N,S
210 IF N=0.0 THEN 490
220 LET H=0.0
230 FOR I=1 TO S
240 READ N(I)
250 LET H=(N(I)/N) * (LOG(N(I)/N)/LOG(2)) + H
260 NEXT I
270 LET H=-H
280 PRINT TAB(2); L; TAB(10); H; TAB(26);
290 IF H<1 THEN 350
300 IF H<3 THEN 330
310 PRINT "CLEAN WATER."
320 GO TO 200
330 PRINT "MODERATE POLLUTION."
340 GO TO 200
350 PRINT  "HEAVY POLLUTION."
360 GO TO 200
370 DATA 1,146,5,131,7,4,1,3,2,181,5,99,68,1,1,12
380 DATA 3,208,6,180,1,18,3,1,5,4,119,4,110,4,1,4
390 DATA 5,401,6,326,17,9,1,6,42,6,596,4,593,1,1,1
400 DATA 9,203,9,118,2,61,11,2,1,1,6,1
410 DATA 10,64,6,48,2,9,2,1,2
420 DATA 11,144,5,131,5,6,1,1,12,72,6,59,7,1,1,3,1
430 DATA 13,335,4,288,15,31,1,14,48,4,35,1,2,10
440 DATA 15,381,6,336,5,30,1,8,2,16,68,4,58,2,3,5
450 DATA 17,509,7,494,3,2,1,2,6,1,18,24,5,8,1,3,1,11
460 DATA 19,523,6,511,1,6,1,2,2,20,94,6,62,3,21,1,2,5
470 DATA 21,263,6,228,9,13,9,3,1,22,80,7,3,2,30,41,1,2,1
480 DATA 0,0,0
490 END
```

4. DPLOT

DPLOT is a teletype plotting program for data. Some programming changes are necessary to alter the scale (at present, the program is set from 0 to 3). Since 60 is a reasonable teletype size (70 is maximum), a factor must be devised by which to multiply the result in line 270 so as to equal 60 (i.e., for a high scale of 3, multiply by 20). It is necessary to change line 390 to indicate the new scale and to divide the result (namely R(I)) by the appropriate multiple in lines 510 and 560.

Input location, name of test, date, remarks (if any), site number, and result at that site. The output will be a scaled graph on which the site numbers and results at each site will be printed.

A "RUN" and "LIST" follows.

```
OLD DPLOT
READY

RUN

DPLOT      09 AUG 70  19:58

LOCATION ? NESHEMINY
TEST RESULTS FOR ? DIVERSITY
DATE TAKEN ? 6-24-69
REMARKS? COMPLETE WATERSHED
NO. OF SITES TESTED ? 20
SITE NO. AND RESULTS? 1,0.6571
SITE NO. AND RESULTS? 2,1.3492
SITE NO. AND RESULTS? 3,0.7776
SITE NO. AND RESULTS? 4,0.4919
SITE NO. AND RESULTS? 5,1.0123
SITE NO. AND RESULTS? 6,0.05365
SITE NO. AND RESULTS? 9,1.5988
SITE NO. AND RESULTS? 10,1.2718
SITE NO. AND RESULTS? 11,0.5831
SITE NO. AND RESULTS? 12,1.0105
SITE NO. AND RESULTS? 13,0.7309
SITE NO. AND RESULTS? 14,1.1111
SITE NO. AND RESULTS? 15,0.7099
SITE NO. AND RESULTS? 16,0.8209
SITE NO. AND RESULTS? 17,0.2592
SITE NO. AND RESULTS? 18,1.8013
SITE NO. AND RESULTS? 19,0.2026
SITE NO. AND RESULTS? 20,1.4507
SITE NO. AND RESULTS? 21,0.8305
SITE NO. AND RESULTS? 22,1.6266
```

```
TEST RESULTS FOR DIVERSITY TAKEN ON 6-24-69 ON NESHEMINY

REMARKS: COMPLETE WATERSHED

     SCALE FROM 0 TO 3.

     ....................+...................+.....,.............
     .
  1  .            *( 0.6571 )
     .
  2  .                         *( 1.3492 )
     .
  3  .              *( 0.7776 )
     .
  4  .        *( 0.4919 )
     .
  5  .                   *( 1.0123 )
     .
  6  .*( 0.05365 )
     .
  9  .                              *( 1.5988 )
     .
 10  .                        *( 1.2718 )
     .
 11  .          *( 0.5831 )
     .
 12  .                   *( 1.0105 )
     .
 13  .             *( 0.7309 )
     .
 14  .                     *( 1.1111 )
     .
 15  .             *( 0.7099 )
     .
 16  .               *( 0.8209 )
     .
 17  .    *( 0.2592 )
     .
 18  .                                  *( 1.8013 )
     .
 19  .   *( 0.2026 )
     .
 20  .                           *( 1.4507 )
     .
 21  .               *( 0.8305 )
     .
 22  .                              *( 1.6266 )


TIME: 0.330 SEC.
READY
```

DPLOT

```
110' PLOT ROUTINE FOR DATA
120'
130 DIM S(30),R(30)
140 PRINT "LOCATION ";
150 INPUT L$
160 PRINT "TEST RESULTS FOR ";
170 INPUT T$
180 PRINT "DATE TAKEN ";
190 INPUT D$
200 PRINT "REMARKS";
210 INPUT R$
220 PRINT "NO. OF SITES TESTED ";
230 INPUT N
240 FOR I=1 TO N
250 PRINT "SITE NO. AND RESULTS";
260 INPUT S(I),R(I)
270 LET R(I)=R(I)*20
280 NEXT I
290 PRINT
300 PRINT
310 PRINT
320 PRINT
330 PRINT "TEST RESULTS FOR ";T$;" TAKEN ON ";D$;" ON ";L$
340 PRINT
350 PRINT "REMARKS: ";R$
360 PRINT
370 PRINT
380 PRINT TAB(5);
390 PRINT "SCALE FROM 0 TO 3."
400 PRINT
410 PRINT TAB(5);
420 FOR I=1 TO 61
430 IF I=21 THEN 580
440 IF I=41 THEN 580
450 PRINT ".";
460 NEXT I
470 PRINT
480 FOR I=1 TO N
490 PRINT TAB(5);"."
500 IF R(I)=0 THEN 560
510 PRINT S(I);TAB(5);".";TAB(R(I)+5);"*";"(";R(I)/20;")"
520 NEXT I
530 PRINT
540 PRINT
550 GO TO 600
560 PRINT S(I);TAB(5);"*";TAB(9);"(";R(I)/20;")"
570 GO TO 520
580 PRINT "+";
590 GO TO 460
600 END
```

5. ANALYZE

ANALYZE is programmed to help analyze a sample of water quickly and accurately. It uses as its parameters: pH, $C0_2$, D0, ID0D, B0D, C0LIF0RM, FECAL C0LIF0RM, FECAL STREP, and PH0SPHATE.

Input location, site number, date, and the above parametric readings obtained from your tests. The output consists of general comments about each parametric result and, in some cases, suggests other tests that might be done to obtain a more complete analysis.

A "RUN" and "LIST" follows.

```
RUN

ANALYZE   09 AUG 70  20:57

THIS PROGRAM IS DESIGNED TO HELP YOU ANALYZE YOUR SAMPLE.

LOCATION? WINNESQUAM RIVER
SITE #? 3
DATE? 3-24-70


DO YOU HAVE A PH READING? YES
READING? 6.2

DO YOU HAVE A CO2 READING? YES
READING? 18

DO YOU HAVE A DISSOLVED OXYGEN (D.O.) READING? YES
READING? 4
TEMP IN C? 13

DO YOU HAVE A READING OF IMMEDIATE DISSOLVED OXYGEN
DEMAND (I.D.O.D.)? YES
READING? 2

DO YOU HAVE A READING OF BIOLOGICAL OXYGEN DEMAND (B.O.D.)? YES
READING? 10

DO YOU HAVE A COLIFORM COUNT PER 100 ML.? YES
READING? 33766

DO YOU HAVE A READING OF FECAL COLIFORM PER 100 ML.? YES
READING? 250

DO YOU HAVE A FECAL STREP READING? YES
READING? 100

DO YOU HAVE A PHOSPHATES READING IN PPM.? YES
READING? 0.02
```

```
RESULTS FROM WINNESQUAM RIVER TAKEN ON 3-24-70 AT SITE  3 .

THE WATER IS NEUTRAL.

CO2 COULD BE A LIMITING FACTOR IF D.O. IS LOW AND
PH IS NOT 'NATURAL'.

D.O. IS LOW FOR CLASS 'A' WATERS.


AT  13  DEGREES C.,THE THEORETICAL D.O. SATURATION LEVEL IS
 10.6 .
THE PERCENT OF D.O. IN RELATION TO THE THEORETICAL
D.O. SATURATION IS 27 %.

THE O2 BALANCE IN THIS WATER IS POOR. CHECK I.D.O.D.,
B.O.D.,COLIFORM COUNT FOR POSSIBLE CLUES AS TO THE REASON.

NOTE: DISSO=D.O.-I.D.O.D.

DISSO OF  2  SHOWS OXYGEN DEMANDING MATERIAL IN THE WATER.

THE READING OF  10  FOR B.O.D. HAS NO DISTINCT
RELATIONSHIP TO D.O. BECAUSE OF A VOLUMETRIC DIFFERENCE.

TOTAL COLIFORMS ARE CONSIDERED 'RELIABLE' INDICATORS
AS TO THE POSSIBLE PRESENCE OF BACTERIAL PATHOGENS...
SINCE THE TOTAL COUNT IS  33766  PER 100ML. ,THIS BODY
OF WATER IS UNFIT FOR HUMAN CONTACT.

THE PRESENCE OF FECAL COLIFORMS IN WATER INDICATES
RECENT FECAL CONTAMINATION. SINCE THE FECAL COUNT WAS
REPORTED AS  250  PER 100 ML., THIS BODY OF WATER IS
UNFIT FOR PUBLIC WATER SUPPLY.

SINCE FECAL STREP EQUALS  100  THE CONTAMINATION
IS LIKELY HUMAN WASTE.

PHOSPHATES ARE PRESENT IN SUFFICIENT AMOUNTS, 0.02
 PPM, THAT COULD 'TRIGGER' AN ALGAL BLOOM,IF OTHER
CONDITIONS ARE RIGHT.


TIME: 0.998 SEC.
READY
```

ANALYZE

```
100'
110' WATER ANALYSIS PROGRAM
120'
130 PRINT
140 PRINT "THIS PROGRAM IS DESIGNED TO HELP YOU ANALYZE YOUR SAMPLE."
150 PRINT
160 PRINT "LOCATION";
170 INPUT L$
180 PRINT "SITE #";
190 INPUT S1
200 PRINT "DATE";
210 INPUT D$
220 MARGIN 65
230 PRINT
240 PRINT
250 PRINT
260 LET I=0
270 DIM A(20),B(60)
280 PRINT "DO YOU HAVE A PH READING";
290 GOSUB 1490
300 PRINT "DO YOU HAVE A CO2 READING";
310 GOSUB 1490
320 PRINT"DO YOU HAVE A DISSOLVED OXYGEN (D.O.) READING";
330 GOSUB 1490
340 PRINT "DO YOU HAVE A READING OF IMMEDIATE DISSOLVED OXYGEN"
350 PRINT "DEMAND (I.D.O.D.)";
360 GOSUB 1490
370 PRINT "DO YOU HAVE A READING OF BIOLOGICAL OXYGEN DEMAND (B.O.D.)";
380 GOSUB 1490
390 PRINT "DO YOU HAVE A COLIFORM COUNT PER 100 ML.";
400 GOSUB 1490
410 PRINT "DO YOU HAVE A READING OF FECAL COLIFORM PER 100 ML.";
420 GOSUB 1490
430 PRINT "DO YOU HAVE A FECAL STREP READING";
440 GOSUB 1490
450 PRINT "DO YOU HAVE A PHOSPHATE  READING IN PPM.";
460 GOSUB 1490
470 REM PH=A(1)  CO2=A(2)  O2=A(3)  IDOD=A(4)  BOD=A(5)  COLIFORM=A(6)
480 REM FECAL COLIFORM=A(7) FECAL STREP=A(8) PHOSPHATES=A(9)
490 PRINT
500 PRINT
510 PRINT "RESULTS FROM ";L$;" TAKEN ON ";D$;" AT SITE ";S1;"."
520 PRINT
530 PRINT
540 IF A(1)=-1 THEN 650
550 IF A(1)>8.6 THEN 640
560 IF A(1)>8.2 THEN 620
570 IF A(1)>5.999  THEN 600
580 PRINT "THE WATER IS ACIDIC."
590 GO TO 650
```

ANALYZE (CONTINUED)

```
600 PRINT "THE WATER IS NEUTRAL."
610 GO TO 650
620 PRINT "THE WATER IS SLIGHTLY ALKALINE."
630 GO TO 650
640 PRINT "THE WATER IS ALKALINE."
650 PRINT
660 IF A(2)=-1 THEN 770
670 IF A(2)>25 THEN 740
680 IF A(2)>15 THEN 710
690 PRINT "CO2 IS NOT A LIMITING FACTOR."
700 GO TO 770
710 PRINT "CO2 COULD BE A LIMITING FACTOR IF D.O. IS LOW AND ";
720 PRINT "PH IS NOT 'NATURAL'."
730 GO TO 770
740 PRINT "CO2 IS PROBABLY A LIMITING FACTOR.EXAMINE THE ";
750 PRINT "AREA WITH A DI-URNAL STUDY FOR POSSIBLE ALGAL ";
760 PRINT "BLOOM.  EXAMINE, ALSO, PH AND D.O. READINGS."
770 PRINT
780 IF A(3)=-1 THEN 1290
790 IF A(3)>8.2 THEN 830
800 IF A(3)>5 THEN 880
810 PRINT "D.O. IS LOW FOR CLASS 'A' WATERS."
820 GO TO 900
830 PRINT "THE D.O. IS HIGHER THAN IS 'NATURAL'.IF THE D.O. IS ";
840 PRINT "GREATER THAN 10, IT MAY INDICATE AN ALGAL BLOOM OR SOME ";
850 PRINT "'UNNATURAL' CONDITION.IT IS RECOMMENDED THAT A ";
860 PRINT "COMPLETE O2 ANALYSIS BE DONE AT THIS SITE."
870 GO TO 900
880 PRINT "D.O. IS PROBABLY NOT A LIMITING FACTOR AND WILL SUPPORT ";
890 PRINT "MOST FISH LIFE."
900 PRINT
910 PRINT
920 LET I=0
930 LET B(I)=14.6
940 FOR I=1 TO 50
950 READ B(I)
960 NEXT I
970 LET P=(A(3)/B(Q))*100
980 PRINT "AT ";T;" DEGREES C.,THE THEORETICAL D.O. SATURATION";
990 PRINT " LEVEL IS ";B(T);"."
1000 PRINT "THE PERCENT OF D.O. IN RELATION TO THE THEORETICAL ";
1010 LET P=INT(P+.5)
1020 PRINT "D.O. SATURATION IS";P;"%."
1030 DATA 14.2,13.8,13.5,13.1,12.8,12.5,12.2,11.9,11.6,11.3,11.1
1040 DATA 10.8,10.6,10.4,10.2,10.0,9.7,9.5,9.4,9.2,9.0,8.8,8.7,8.5
1050 DATA 8.4,8.2,8.1,7.9,7.8,7.6,7.5,7.4,7.3,7.2,7.1,7.0,6.9
1060 DATA 6.8,6.7,6.6,6.5,6.4,6.3,6.2,6.1,6.0,5.9,5.8,5.7,5.6
1070 IF P>=75 THEN 1170
1080 IF P>=50 THEN 1130
1090 PRINT
```

```
1100 PRINT "THE O2 BALANCE IN THIS WATER IS POOR. CHECK I.D.O.D.,";
1110 PRINT "B.O.D.,COLIFORM COUNT FOR POSSIBLE CLUES AS TO THE REASON."
1120 GO TO 1290
1130 PRINT
1140 PRINT " THE O2 BALANCE IS FAIR. IT PROBABLY DOES NOT ACT AS A ";
1150 PRINT "LIMITING FACTOR,ESPECIALLY IF THE TEMP IS <15 CENT."
1160 GO TO 1290
1170 PRINT
1180 IF P>100 THEN 1250
1190 PRINT "THE O2 BALANCE IS GOOD,AND SHOULD INDICATE A HEALTHY ";
1200 PRINT "STREAM. NO ANAEROBIC CONDITIONS SHOULD BE PRESENT IF ";
1210 PRINT "A REPRESENTATIVE SAMPLE WAS TAKEN...CAUTION! CHECK ";
1220 PRINT "FLOW READINGS FOR THEY MAY MASK ACYUAL CONDITIONS."
1230 GO TO 1290
1240 PRINT
1250 PRINT "O2 FIGURES INDICATE A 'SUPER-SATURATED' CONDITION.";
1260 PRINT "RECHECK O2 READING. A SPECIES DIVERSITY IS RECOMMENDED ";
1270 PRINT "- ALONG WITH A COMPLETE ALGAL COUNT..."
1280 PRINT
1290 IF A(4)=-1 THEN 1440
1300 IF A(4)>A(3) THEN 1430
1310 IF A(4)<.5 THEN 1430
1320 LET D1=A(3)-A(4)
1330 PRINT
1340 PRINT "NOTE: DISSO=D.O.-I.D.O.D."
1350 PRINT
1360 IF D1+3<0 THEN 1400
1370 PRINT "DISSO OF ";D1;" SHOWS OXYGEN DEMANDING MATERIAL IN THE ";
1380 PRINT "WATER."
1390 GO TO 1440
1400 PRINT "DISSO OF ";D1;" SHOWS OXYGEN DEMANDING MATERIALS IN THE ";
1410 PRINT "WATER ARE NOT A FACTOR."
1420 GO TO 1440
1430 GOSUB 1650
1440 IF A(5)=-1 THEN 1710
1450 PRINT
1460 PRINT "THE READING OF ";A(5);" FOR B.O.D. HAS NO DISTINCT ";
1470 PRINT "RELATIONSHIP TO D.O. BECAUSE OF A VOLUMETRIC DIFFERENCE."
1480 GO TO 1710
1490 INPUT R$
1500 LET I=I+1
1510 IF R$="NO" THEN 1620
1520 IF R$="YES" THEN 1580
1530 PRINT "INCORRECT FORMAT, PLEASE TYPE YES OR NO."
1540 PRINT "DO YOU HAVE A READING";
1550 INPUT R$
1560 GO TO 1510
1570 PRINT
1580 PRINT "READING";
1590 INPUT A(I)
```

ANALYZE (CONTINUED)

```
1600 IF I=3 THEN 1650
1610 GO TO 1630
1620 LET A(I)=-1
1630 PRINT
1640 RETURN
1650 PRINT "TEMP IN C";
1660 INPUT T
1670 PRINT
1680 GO TO 1640
1690 PRINT "THESE RESULTS FOR I.D.O.D. ARE IMPOSSIBLE,RECHECK."
1700 RETURN
1710 IF A(6)<=50 THEN 1750
1720 PRINT
1730 IF A(6)<250 THEN 1800
1740 IF A(6)>=250 THEN 1880
1750 IF A(6)=-1 THEN 2310
1760 PRINT "THE TOTAL COLIFORM COUNT IS ";A(6);" PER 100 ML.";
1770 PRINT " THE COUNT IS SUFFICIENT FOR CLASS 'A' WATER."
1780 PRINT
1790 GO TO 1930
1800 PRINT "TOTAL COLIFORM COUNT IS CONSIDERED A 'RELIABLE' INDICATOR ";
1810 PRINT "AS TO THE POSSIBLE PRESENCE OF BACTERIAL PATHOGENS ...";
1820 PRINT "THEIR PRESENCE INDICATES INADEQUATE WASTE TREATMENT.";
1830 PRINT "SINCE THE TOTAL COUNT IS ";A(6);" PER 100 ML., THIS ";
1840 PRINT "BODY OF WATER IS LIMITED TO BATHING AND IS UNACCEPTABLE ";
1850 PRINT "FOR PUBLIC WATER SUPPLY."
1860 PRINT
1870 GO TO 1930
1880 PRINT "TOTAL COLIFORMS ARE CONSIDERED 'RELIABLE' INDICATORS ";
1890 PRINT "AS TO THE POSSIBLE PRESENCE OF BACTERIAL PATHOGENS...";
1900 PRINT "SINCE THE TOTAL COUNT IS ";A(6);" PER 100ML. ,THIS BODY ";
1910 PRINT "OF WATER IS UNFIT FOR HUMAN CONTACT."
1920 PRINT
1930 IF A(7)>0 THEN 2000
1940 IF A(7)=-1 THEN 2060
1950 PRINT "THIS BODY OF WATER PROBABLY HAS NO RECENT FECAL ";
1960 PRINT "CONTAMINATION. HOWEVER, IF THE TOTAL COLIFORM COUNT ";
1970 PRINT "IS >50 PER 100 ML., ANOTHER FECAL COUNT IS ADVISED."
1980 PRINT
1990 GO TO 2060
2000 PRINT "THE PRESENCE OF FECAL COLIFORMS IN WATER INDICATES ";
2010 PRINT "RECENT FECAL CONTAMINATION. SINCE THE FECAL COUNT WAS ";
2020 PRINT "REPORTED AS ";A(7);" PER 100 ML., THIS BODY OF WATER IS ";
2030 PRINT "UNFIT FOR PUBLIC WATER SUPPLY."
2040 PRINT
2050 GOTO 2210
2060 IF A(9)<0.015 THEN 2120
2070 PRINT "PHOSPHATES ARE PRESENT IN SUFFICIENT AMOUNTS,";A(9);
2080 PRINT " PPM, THAT COULD 'TRIGGER' AN ALGAL BLOOM,IF OTHER ":
2090 PRINT "CONDITIONS ARE RIGHT."
```

ANALYZE (CONTINUED)

```
2100 PRINT
2110 GO TO 2310
2120 PRINT "PHOSPHATES,REPORTED TO BE PRESENT IN QUANTITIES OF ";A(9);
2130 PRINT " PPM, SEEM SUFFICIENTLY LOW TO PARTIALLY INHIBIT ";
2140 PRINT "THE PRODIGIOUS GROWTHS OF ALGAE. IF ALGAE DOES PERSIST ";
2150 PRINT "IN LARGE NUMBERS, CHECK THE FOLLOWING:"
2160 PRINT TAB(13);"NITRATE-NITROGEN"
2170 PRINT TAB(13);"NITRITE-NITROGEN"
2180 PRINT TAB(13);"AMMONIA COMPOUNDS"
2190 PRINT
2200 GO TO 2310
2210 IF A(8)=-1 THEN 2060
2220 IF A(7)/A(8)>1 THEN 2270
2230 PRINT "SINCE FECAL STREP EQUALS ";A(8);" THE CONTAMINATION ";
2240 PRINT "IS LIKELY ANIMAL WASTE."
2250 PRINT
2260 GO TO 2060
2270 PRINT "SINCE FECAL STREP EQUALS ";A(8);" THE CONTAMINATION ";
2280 PRINT "IS LIKELY HUMAN WASTE."
2290 PRINT
2300 GO TO 2060
2310 END
```

6. STR-CLAS

STR-CLAS is a stream classification program using New Hampshire standards established January 1, 1970.* See Table 1 at end of this appendix. The parameters for the classification are: pH, DØ, TURBIDITY, HYDRØCARBON (ØIL), CØLIFØRM, FECAL CØLIFØRM, AND FECAL STREP. By inputting the above parameters from any site study, the output will consist of a classification (A,B,C,D) per parameter. The lowest classification being the stream classification at that site.

A subprogram, "STREAM 1," calculates volume of flow and another subprogram, "SATTABLE," provides a table of DO saturations from 0° to 50° C for checking your results. A basic description of the final classification is also available.

A "RUN" and "LIST" follows.

---

* This program will be revised to use Federal Standards.

```
RUN

STR-CLAS  09 AUG 70  20:46

 THIS PROGRAM CLASSIFIES WATER PER SITE BY N.H.STANDARDS.

LOCATION ? WINNESQUAM RIVER
DATE OF TEST ? 7-24-70
SITE # ? 3

DO YOU HAVE A PH READING? YES
READING? 6.2

DO YOU HAVE A DISSOLVED OXYGEN (D.O.) READING? YES
READING? 4
WATER TEMP IN C? 13

DO YOU HAVE A TURBIDITY READING? YES
READING? 22

DO YOU HAVE A HYDROCARBON (OIL) FACTOR? NO

DO YOU HAVE A COLIFORM COUNT PER ML.? YES
READING? 33766

DO YOU HAVE A FECAL COLIFORM READING? YES
READING? 250

DO YOU HAVE A FECAL STREP READING? YES
READING? 100

DO YOU HAVE A FLOW CALCULATION TO MAKE? NO
DO YOU HAVE THE VOLUMETRIC VALUE ALREADY? YES
VALUE? 385.1
```

```
                    **********


SOURCE: NEW HAMPSHIRE WATER SUPPLY AND POLLUTION CONTROL
COMMISSION.         JAN.1,1970.

NOTE: THE FINAL CLASSIFICATION IS THE LOWEST CLASS NOTED BELOW!

RESULTS OF WATER CLASSIFICATION FOR SITE  3  ON WINNESQUAM RIVER FOR
7-24-70


A PH OF  6.2  INDICATES CLASS C WATER.

A D.O. OF  4  INDICATES CLASS D WATER.

A TURBIDITY READING OF  22  INDICATES CLASS B OR C WATER.

     COMMENT:NON-TROUT STREAM ACCEPTABILITY.

A TOTAL COLIFORM READING OF  33766  PER 100 ML.
INDICATES CLASS C OR D WATER.


               COMMENT:
     ANY FECAL COLIFORM OR FECAL STREP READINGS ( YOURS
     ARE  250  AND  100 ) INDICATES RECENT
     CONTAMINATION BY WARM BLOODED ANIMALS.
     THIS CONTAMINATION IS LIKELY HUMAN WASTE.

ALL OF THIS IS BEING CARRIED ALONG AT  385.1  FT↑3/SEC.




DO YOU WISH AN EXPLAINATION OF THE WATER CLASSES (YES/NO)? YES

WHICH CLASS OF WATER (A,B,C,D)? C

 CLASS C: ACCEPTABLE FOR RECREATIONAL BOATING,FISHING,
 AND INDUSTRIAL WATER SUPPLY WITH OR WITHOUT TREATMENT,
 DEPENDING ON INDIVIDUAL REQUIREMENTS. ( THIRD HIGHEST
 QUALITY).

WOULD YOU LIKE ANOTHER CLASS EXPLAINED (YES/NO)? YES
WHICH CLASS OF WATER (A,B,C,D)? D

 CLASS D: AESTHETICALLY ACCEPTABLE. SUITABLE FOR CERTAIN
 INDUSTRIAL PURPOSES, POWER AND NAVIGATION. (LOWEST ALL-
 OWABALE QUALITY NOW LESS THAN 1/2 MILE IN ENTIRE STATE).

WOULD YOU LIKE ANOTHER CLASS EXPLAINED (YES/NO)? NO

DO YOU WISH A TABLE OF THEORETICAL D.O.SATURATION FROM 0 TO 50 CENT.? YE
```

THEORETICAL D.O.SATURATION TABLE FROM 0 TO 50 CENT.

TEMP (^{o}C)	THEORETICAL D.O. (ppm)
0	14.6
1	14.2
2	13.8
3	13.5
4	13.1
5	12.8
6	12.5
7	12.2
8	11.9
9	11.6
10	11.3
11	11.1
12	10.8
13	10.6
14	10.4
15	10.2
16	10
17	9.7
18	9.5
19	9.4
20	9.2
21	9
22	8.8
23	8.7
24	8.5
25	8.4
26	8.2
27	8.1
28	7.9
29	7.8
30	7.6
31	7.5
32	7.4
33	7.3
34	7.2
35	7.1
36	7
37	6.9
38	6.8
39	6.7
40	6.6
41	6.5
42	6.4
43	6.3
44	6.2
45	6.1
46	6
47	5.9
48	5.8
49	5.7
50	5.6

STR-CLAS

```
100 PRINT
110 SUB STREAM1;SATTABLE
120 MARGIN 70
130 '
140 ' WATER CLASSIFICATION PROGRAM FOR NEW HAMPSHIRE.
150 '
160 ' WRITTEN DURING WATER POLLUTION PROGRAM - SUMMER '70
170 PRINT " THIS PROGRAM CLASSIFIES WATER PER SITE BY N.H.STANDARDS."
180 PRINT
190 PRINT
200 PRINT "LOCATION ";
210 INPUT L$
220 PRINT "DATE OF TEST ";
230 INPUT D$
240 PRINT "SITE # ";
250 INPUT S1
260 LET I=0
270 PRINT
280 PRINT "DO YOU HAVE A PH READING";
290 GOSUB 2510
300 PRINT "DO YOU HAVE A DISSOLVED OXYGEN (D.O.) READING";
310 GOSUB 2510
320 PRINT "DO YOU HAVE A TURBIDITY READING";
330 GOSUB 2510
340 PRINT"DO YOU HAVE A HYDROCARBON (OIL) FACTOR";
350 GOSUB 2510
360 PRINT "DO YOU HAVE A COLIFORM COUNT PER ML.";
370 GOSUB 2510
380 PRINT "DO YOU HAVE A FECAL COLIFORM READING";
390 GOSUB 2510
400 PRINT "DO YOU HAVE A FECAL STREP READING";
410 GOSUB 2510
420 PRINT "DO YOU HAVE A FLOW CALCULATION TO MAKE";
430 INPUT R$
440 IF R$="YES" THEN 510
450 PRINT "DO YOU HAVE THE VOLUMETRIC VALUE ALREADY";
460 INPUT A$
470 IF A$="NO" THEN 530
480 PRINT "VALUE";
490 INPUT F
500 GO TO 530
510 GOSUB #1
520 '
530 ' A(1)=PH   A(2)=D.O.   A(3)=TURBIDITY   A(4)=OIL   A(5)=COLIFORM
540 ' A(6)=FECAL COLIFORM   A(7)=FECAL STREP
550 '
560 LET I=1
570 IF A(I)=-1 THEN 710
580 IF A(I)>8.5 THEN 640
590 IF A(I)>8 THEN 660
```

STR-CLAS (CONTINUED)

```
600 IF A(I)>6.5 THEN 680
610 IF A(I)>6 THEN 700
620 GOSUB 2200
630 GO TO 710
640 GOSUB 2200
650 GO TO 710
660 GOSUB 2180
670 GO TO 710
680 GOSUB 2220
690 GO TO 710
700 GOSUB 2180
710 LET I=2
720 IF A(I)=-1 THEN 870
730 FOR J=0 TO 50
740 READ S(J)
750 NEXT J
760 DATA 14.6,14.2,13.8,13.5,13.1,12.8,12.5,12.2,11.9,11.6,11.3,11.1
770 DATA 10.8,10.6,10.4,10.2,10.0,9.7,9.5,9.4,9.2,9.0,8.8,8.7,8.5
780 DATA 8.4,8.2,8.1,7.9,7.8,7.6,7.5,7.4,7.3,7.2,7.1,7.0,6.9
790 DATA 6.8,6.7,6.6,6.5,6.4,6.3,6.2,6.1,6.0,5.9,5.8,5.7,5.6,5.5
800 IF A(I)>.75*S(T) THEN 840
810 IF A(I)> 5 THEN 860
820 GOSUB 2200
830 GO TO 870
840 GOSUB 2220
850 GO TO 870
860 GOSUB 2180
870 LET I=3
880 IF A(I)=-1 THEN 1010
890 IF A(I)<5 THEN 940
900 IF A(I)<10 THEN 960
910 IF A(I)<25 THEN 990
920 GOSUB 2200
930 GO TO 1010
940 GOSUB 2140
950 GO TO 1010
960 GOSUB 2240
970 GOSUB 2280
980 GO TO 1010
990 GOSUB 2240
1000 GOSUB 2300
1010 LET I=4
1020 IF A(I)=-1 THEN 1110
1030 IF A(I)=1 THEN 1080
1040 IF A(I)=2 THEN 1100
1050 PRINT "INPUT EITHER 1 OR 2 FOR THE OIL FACTOR";
1060 INPUT A(I)
1070 GO TO 1030
1080 GOSUB 2180
1090 GO TO 1110
```

STR-CLAS (CONTINUED)

```
1100 GOSUB 2200
1110 LET I=5
1120 IF A(I)=-1 THEN 1200
1130 IF A(I)<50 THEN 1170
1140 IF A(I)<240 THEN 1190
1150 GOSUB 2260
1160 GO TO 1200
1170 GOSUB 2140
1180 GO TO 1200
1190 GOSUB 2160
1200 PRINT
1210 PRINT TAB(25);
1220 FOR I=1 TO 10
1230 PRINT "*";
1240 NEXT I
1250 FOR I=1 TO 4
1260 PRINT
1270 NEXT I
1280 PRINT "SOURCE: NEW HAMPSHIRE WATER SUPPLY AND POLLUTION CONTROL"
1290 PRINT "COMMISSION.          JAN.1,1970."
1300 PRINT
1310 PRINT "NOTE: THE FINAL CLASSIFICATION IS THE LOWEST CLASS NOTED";
1320 PRINT " BELOW!"
1330 PRINT
1340 PRINT "RESULTS OF WATER CLASSIFICATION FOR SITE ";S1;" ON ";
1350 PRINT L$; " FOR ";D$
1360 PRINT
1370 PRINT
1380 IF A(1)=-1 THEN 1410
1390 PRINT "A PH OF ";A(1);" INDICATES CLASS ";A$(1);" WATER."
1400 PRINT
1410 IF A(2)=-1 THEN 1440
1420 PRINT "A D.O. OF ";A(2);" INDICATES CLASS ";A$(2);" WATER."
1430 PRINT
1440 IF A(3)=-1 THEN 1500
1450 PRINT "A TURBIDITY READING OF ";A(3);" INDICATES CLASS ";A$(3);
1460 PRINT " WATER."
1470 IF A(3)<10 THEN 2110
1480 IF A(3)<25 THEN 2110
1490 PRINT
1500 IF A(4)=-1 THEN 1590
1510 IF A(4)=1 THEN 1560
1520 PRINT "A DEFINITE HYDROCARBON (OIL) OBSERVATION INDICATES CLASS";
1530 PRINT " D WATER."
1540 PRINT
1550 GO TO 1590
1560 PRINT "A SLIGHT HYDROCARBON (OIL) FILM INDICATES AT BEST CLASS";
1570 PRINT " C WATER."
1580 PRINT
1590 IF A(5)=-1 THEN 1630
```

STR-CLAS (CONTINUED)

```
1600 PRINT "A TOTAL COLIFORM READING OF ";A(5);" PER 100 ML."
1610 PRINT "INDICATES CLASS ";A$(5);" WATER."
1620 PRINT
1630 IF A(6)=-1 THEN 1740
1640 PRINT
1650 PRINT TAB(15);"COMMENT:"
1660 PRINT TAB(5);"ANY FECAL COLIFORM OR FECAL STREP READINGS ( YOURS"
1670 PRINT TAB(5);"ARE ";A(6);" AND ";A(7);") INDICATES RECENT"
1680 PRINT TAB(5);"CONTAMINATION BY WARM BLOODED ANIMALS."
1690 IF A(7)=-1 THEN 1740
1700 IF A(6)/A(7)>1 THEN 1730
1710 PRINT TAB(5);"THIS CONTAMINATION IS LIKELY ANIMAL WASTE."
1720 GO TO 1740
1730 PRINT TAB(5);"THIS CONTAMINATION IS LIKELY HUMAN WASTE."
1740 PRINT
1750 PRINT "ALL OF THIS IS BEING CARRIED ALONG AT ";F;" FT↑3/SEC."
1760 FOR J=1 TO 5
1770 PRINT
1780 NEXT J
1790 PRINT "DO YOU WISH AN EXPLAINATION OF THE WATER CLASSES (YES/NO)";
1800 INPUT R$
1810 IF R$="YES" THEN 1830
1820 GO TO 2030
1830 PRINT
1840 PRINT "WHICH CLASS OF WATER (A,B,C,D)";
1850 INPUT L$
1860 IF L$="A" THEN 1910
1870 IF L$="B" THEN 1940
1880 IF L$="C" THEN 1970
1890 GOSUB 2320
1900 GO TO 1990
1910 PRINT
1920 GOSUB 2370
1930 GO TO 1990
1940 PRINT
1950 GOSUB 2410
1960 GO TO 1990
1970 PRINT
1980 GOSUB 2460
1990 PRINT
2000 PRINT "WOULD YOU LIKE ANOTHER CLASS EXPLAINED (YES/NO)";
2010 INPUT R$
2020 IF R$="YES" THEN 1840
2030 PRINT
2040 PRINT "DO YOU WISH A TABLE OF THEORETICAL D.O.SATURATION FROM";
2050 PRINT " 0 TO 50 CENT.";
2060 INPUT A$
2070 IF A$="YES" THEN 2090
2080 STOP
2090 GOSUB #2
```

STR-CLAS (CONTINUED)

```
2100 STOP
2110 PRINT
2120 PRINT TAB(5);"COMMENT:";B$(3)
2130 GO TO 1490
2140 LET A$(I)="A"
2150 RETURN
2160 LET A$(I)="B"
2170 RETURN
2180 LET A$(I)="C"
2190 RETURN
2200 LET A$(I)="D"
2210 RETURN
2220 LET A$(I)="A OR B"
2230 RETURN
2240 LET A$(I)="B OR C"
2250 RETURN
2260 LET A$(I)="C OR D"
2270 RETURN
2280 LET B$(I)="TROUT STREAM ACCEPTABILITY."
2290 RETURN
2300 LET B$(I)="NON-TROUT STREAM ACCEPTABILITY."
2310 RETURN
2320 PRINT
2330 PRINT " CLASS D: AESTHETICALLY ACCEPTABLE. SUITABLE FOR CERTAIN"
2340 PRINT " INDUSTRIAL PURPOSES, POWER AND NAVIGATION. (LOWEST ALL-"
2350 PRINT " OWABALE QUALITY NOW LESS THAN 1/2 MILE IN ENTIRE STATE)."
2360 RETURN
2370 PRINT " CLASS A: POTENTIALLY ACCEPTABLE FOR PUBLIC WATER SUPPLY"
2380 PRINT " AFTER DISINFECTION. NO DISCHARGE OF SEWAGE OR OTHER"
2390 PRINT " WASTES. (QUALITY UNIFORMLY EXCELLENT)."
2400 RETURN
2410 PRINT "CLASS B: ACCEPTABLE FOR BATHING AND RECREATION,FISH"
2420 PRINT "HABITAT AND PUBLIC WATER SUPPLY AFTER ADEQUATE TREATMENT."
2430 PRINT " NO DISPOSAL OF SEWAGE OR WASTES UNLESS ADEQUATELY TREATED."
2440 PRINT " (HIGH AESTHETIC VALUE.)"
2450 RETURN
2460 PRINT " CLASS C: ACCEPTABLE FOR RECREATIONAL BOATING,FISHING,"
2470 PRINT " AND INDUSTRIAL WATER SUPPLY WITH OR WITHOUT TREATMENT,"
2480 PRINT " DEPENDING ON INDIVIDUAL REQUIREMENTS. ( THIRD HIGHEST"
2490 PRINT " QUALITY)."
2500 RETURN
2510 LET I=I+1
2520 INPUT R$
2530 IF R$="NO " THEN 2640
2540 IF R$="YES" THEN 2590
2550 PRINT "INCORRECT FORMAT. PLEASE TYPE YES OR NO."
2560 PRINT " DO YOU HAVE A READING";
2570 INPUT R$
2580 GO TO 2530
2590 IF I=4 THEN 2700
```

STR-CLAS (CONTINUED)

```
2600 PRINT "READING";
2610 INPUT A(I)
2620 IF I=2 THEN 2670
2630 GO TO 2650
2640 LET A(I)=-1
2650 PRINT
2660 RETURN
2670 PRINT "WATER TEMP IN C";
2680 INPUT T
2690 GO TO 2650
2700 PRINT "(1) SLIGHT OIL FILM OR (2) OIL SLICK";
2710 INPUT A(I)
2720 GO TO 2650
2730 END
```

STREAM1

```
100 DIM D(20),H(20),I(20)
110 PRINT
120 PRINT
130 PRINT TAB(15);"STREAM CROSSECTION CALCULATION."
140 PRINT
150 PRINT
160 PRINT "WIDTH OF STREAM (F,I)";
170 INPUT W,W5
180 LET W=W+W5/12
190 PRINT "HOW MANY DEPTH READINGS WERE TAKEN";
200 INPUT R
210 PRINT"DISTANCE FROM SHORE TO FIRST MEASUREMENT(FT.) AND";
220 PRINT " DEPTH (F,I)";
230 INPUT D(1),H(1),I(1)
240 LET H(1)=H(1)+I(1)/12
250 LET W1=D(1)
260 FOR J=2 TO R
270 PRINT "DISTANCE(F),DEPTH(F,I)";
280 INPUT D(J),H(J),I(J)
290 LET H(J)=H(J)+I(J)/12
300 LET W1=W1+D(J)
310 NEXT J
320 PRINT "DISTANCE FROM LAST DEPTH TO SHORE";
330 INPUT D(R+1)
340 LET W1=W1+D(R+1)
350 IF W1=W THEN 430
360 PRINT
370 PRINT "ERROR IN WIDTH MEASUREMENT."
380 PRINT
390 PRINT "DO YOU WISH TO CONTINUE";
400 INPUT R$
410 IF R$="YES" THEN 430
420 STOP
430 LET T=.5*D(1)*H(1)
440 LET W=W1
450 FOR I=1 TO R-1
460 IF H(I+1)>H(I) THEN 500
470 LET T=T+.5*(H(I)-H(I+1))*D(I+1)+D(I+1)*H(I+1)
480 NEXT I
490 GO TO 520
500 LET T=T+.5*(H(I+1)-H(I))*D(I+1)+H(I)*D(I+1)
510 GO TO 480
520 LET T=T+.5*D(R+1)*H(R)
530'VELOCITY
540 PRINT
550 PRINT TAB(15);"AVERAGE VELOCITY CALCULATION."
560 PRINT
570 PRINT "HOW MANY TRIALS WERE CONDUCTED";
580 INPUT K
590 LET V=0
```

STREAM1 (CONTINUED)

```
600 FOR J=1 TO K
610 PRINT "DISTANCE BETWEEN POINTS(FT.,IN.)";
620 INPUT F(J),I(J)
630 LET F(J)=F(J)+I(J)/12
640 PRINT "TIME OF FLOAT(SEC.)";
650 INPUT S(J)
660 LET V=F(J)/S(J)+V
670 NEXT J
680 LET V=V/K
690 PRINT "WAS THE STREAM 1)SMOOTH OR 2) ROUGH BOTTOMED";
700 INPUT A
710 IF A=2 THEN 740
720 LET V=V*.9
730 GO TO 750
740 LET V=V*.8
750 LET F=T*V
760 LET F=INT(F+.5)
770 PRINT
780 PRINT "FOR A MORE COMPLETE ANALYSIS,USE THIS DATA IN 'STREAM'."
790 PRINT
800 RETURN
810 END
```

```
SATTABLE

100 DIM S(51)
110 ' THEORETICAL D.O. SATURATION TABLE.
120 PRINT
130 PRINT
140 PRINT "THEORETICAL D.O.SATURATION TABLE FROM 0 TO 50 CENT."
150 PRINT
160 PRINT TAB(26);"THEORETICAL"
170 PRINT TAB(5);"TEMP";TAB(30);"D.O."
180 PRINT
190 FOR I=0 TO 50
200 IF I>9 THEN 260
210 PRINT TAB(6);I;
220 IF S(I)<10 THEN 280
230 PRINT TAB(28);S(I)
240 NEXT I
250 RETURN
260 PRINT TAB(5);I;
270 GO TO 220
280 PRINT TAB(29);S(I)
290 GO TO 240
300 END
```

Appendix 2

Implementation

The implementation of the activities depend on the school's ability to handle several problems, namely: cost, scheduling, and motivation. These problems are interrelated at most schools; therefore, they must be resolved as the program proceeds. Beginning with a small group of interested administrators, teachers, and students seems to be a good approach. A club or single course offering may be as far as a school can proceed in the first year. The schools listed in the preface have programs underway or are beginning programs. Furthermore, they are grouped into clusters which meet monthly, carry on interschool activities, and support the implementation of the program in their region. The organized regions are: Central New Hampshire, Southern New Hampshire-Vermont, Eastern Massachusetts, Central Massachusetts, Northern Massachusetts, New York-Pennsylvania-New Jersey, Washington (D.C.), and South Carolina-Georgia. Any school listed in the preface may be contacted to obtain the cluster coordinator's name. The sections below deal with the various problems of implementation.

A. Cost

The expense involved in beginning a program can be minimized in several ways; costs are increased by equipment, travel, and books and references. Equipment for all but a few activities is not specialized and probably already exists in the various school laboratories. For example, any of the chemical analyses may be made in the lab. Usually, however, this makes testing slow, inconvenient, and remote for the test site. Field test kits which are modular can be obtained to perform selected tests; an elementary kit which contains tests for DO, temperature, turbidity, pH, chlorine, phosphate, sulphate, and nitrate are sufficient to test. Bacterial studies do require equipment which may not be on hand; consult with the staff of your local hospital, county health department, and sewage treatment plant. Usually an arrangement may be worked out to use their facilities.

If your school is a nonprofit enterprise it qualifies for U. S. government surplus. To obtain information on the location of such surplus depots, contact your state education department. Local industry has been found to be particularly helpful; money may not be too easily obtained, but often slightly used equipment or materials will be made available. If you make your needs known through news media and at community meetings and fairs, almost anything can happen. Students at Germantown Academy have helicopter service available for aerial photography as a result of their activities in mapping and monitoring the Wissahickon Creek.

Much of the equipment listed in the activities may be made by hand. If students and teachers work together on this project, a great deal of equipment may be made in a short time. The making of equipment leads to new and better methods of inquiry.

B. Scheduling

Teachers have noted that informal arrangements with other teachers allow the greatest flexibility when the school is unable to provide adequate scheduled time. The sports department arrangements for team games usually may be duplicated if the importance of academic pursuits can be established. Past experience indicates that as the school gains recognition in the community for its achievements, the difficulty in getting time for activities diminishs rapidly. Activities such as (G) and (H) in Chapter 4 are good beginning places to arouse community interest.

C. Motivation

Helping to build the necessary interest in pollution among teachers, students, administrators, and parents will be an important role which you must perform. The pollution problems which we face today are, in themselves, grave enough to motivate people to action. Therefore, in many cases, all that needs to be supplied is a vehicle for expressing and focusing this action. This can very often be accomplished through a club, the planning and presentation of an assembly program, or a variation of an earth day program.

Any of the above activities may serve to allow the students, faculty, administration, and even the parents to become involved and interested in some particular facet of our pollution problems. Once interest and activity have begun there are several means of aiding in its continuance.

School administrators are particularly fond of anything which gains favorable publicity for the school. Most of the above mentioned activities will produce this in the form of newspaper articles and radio and television newscasts. All that you need do is be sure to advise them of what you are planning.

We have found that one factor which aids in the sustained effort of the students is to make them aware of your belief that they are capable of making significant contributions to the community at large. They do possess the initiative and curiosity to determine problems, conduct research, and translate the information into meaningful conclusions, but it is important that you make your awareness of this known to them. A published statement to this effect on the part of the faculty, the publication of a student journal, or the submission of their data and conclusions to the appropriate public agencies serves to substantiate this in the minds of the students.

Since students enjoy this activity-oriented approach and, at the same time, learn more through it, other teachers will want to know what you are doing. Do not be afraid to explain it to them or even better, invite them to participate in the activities along with the students. Your success should provide the necessary motivation for them to try it also.

Appendix 2

It is wise to motivate as many parents as possible. Naturally, they are curious about what their children are doing in school. Invite them to participate or at least arrange for a demonstration for them. They may prove to be an important resource for you in terms of the information, equipment, and transportation which they may be able to provide.

Appendix 3

Limitations

There are a number of possible trouble spots which may pop up during the implementation of these activities. A foreknowledge of these problem areas will, in most cases, be all that is needed to avoid them. In general the problems seem to fall into three categories: time and transportation, methods and equipment, and dealing with other people.

A. Time and Transportation

1. Most activities in thie guide are designed to last an hour and a half; however, many do require more time for completion. For one of the filming activities, for example, students camped overnight on Cardigan Mountain in order to film the sunrise.
2. Some lab experiments required by these activities take a long time to obtain the results. The BOD test, for example, requires 5 days before final results can be obtained. Thus it may become a test of your students' patience.
3. Some people will have difficulty locating suitable streams or lakes for their activities. Streams with the easily monitored depth, rate of flow, width, suitable access points, etc., are not always close to the school.
4. Transporting a large class to and from a site may pose problems in simply obtaining permission to use school busses and to miss lunch, parents' permissions, a driver for the school bus, box lunches, or money for lunches. Methods must be devised to overcome such obstacles. Often making friends with the janitors, cafeteria people, and bus drivers is the key.

B. Methods and Equipment

1. After gaining permission to leave school with your class and having organized all these details, do not forget to advise parents that the students may be required to wade in streams and grovel in the dirt. Students' clothes may become somewhat soiled.
2. Also before leaving the school, be sure you have all the equipment necessary for the activity. This reduces transportation costs and total time required.
3. Students should be well versed in the care of equipment in the field since it is much more difficult to keep it dry and clean.
4. When sterile procedures are required in the field, special care must be taken because the chances of contamination seem to increase in proportion to the distance from the lab.

5. There is some danger involved when students are dealing with sewage wastes. Hip boots and rubber gloves are sometimes a necessity.

6. We have found some keys especially difficult to use. Keep looking for one that is not as vague as the one you are presently using.

7. If the cost of some of the equipment is prohibitive in your case, please use Appendix 2 on Implementation to help remedy these difficulties.

C. Dealing with Others

1. When taking relatively large groups to confined places (a city manager's office, for example) or a privately owned area (such as a farm), it is advisable that the person or persons being contacted understand the size and age level of the group beforehand so that they will be prepared.

2. Always be sure to arrange your appointments well in advance.

3. If you arrange an interview, be sure to list your questions before you arrive. In general, people are willing to cooperate but their time is limited.

4. Always gain permission to use private land, even if you are only using it as access to a lake, river, or stream.

5. Be sure to arrange for the protection of equipment if it must be left in a certain place for any length of time. Many experiments have been disrupted or completely destroyed by curious or unknowing outsiders. Experience has shown that experiments placed in driveways or parking lots are particularly vulnerable.

6. Since these activities are concerned with water pollution, you must be prepared at some point to deal with the polluters themselves. To what extent you are willing to incur their wrath is not only a test of your own moral fortitude but also that of your school.

Appendix 4

Evaluation

Accountability is an essential ingredient in all educational programs. Effective fulfillment of this parameter requires careful planning accompanied by appropriate evaluation. This may seem antagonistic to an approach consisting of activities which are defined only to the point necessary to initiate thinking processes and promulgated through questions which may lead to unexpected outcomes. However, planning and evaluation are "musts" if any success or measure of success is to be obtained.

Organizational and evaluative aids are included in each section of the activity outlines. The introductions state general objectives. The questions provide direction (but not in a dictational manner) and suggest behavioral changes which might be observed for evaluative purposes. Procedures, past examples, and limitations include guidance in organizing the activity.

The foundation for planning and evaluation must begin at the time the activity is selected. This is accomplished by identifying the desired outcomes or objectives on paper in behavioral form. General statements such as the following are of limited use:

a. The student will understand the effects of chemicals on a specific ecosystem.

b. The students will develop an appreciation of the economic factors associated with pollution.

However, behavioral statements which not only designate the desired outcome but also identify a resulting behavior and the conditions under which it is to be observed and evaluated can be extremely useful in planning and evaluative efforts. For example:

> When provided alkalinity, iron, and dissolved oxygen data for a body of water, the student correctly describes one or more biological effect which might be observed at the site.

These objectives should include not only cognitive or psychomotor categories but also affective behavior. While the former are significant, the urgency of current pollution justifies emphasis of attitudes, values, and motivation. Other than these remarks, it is not within the scope of this guide to cover thoroughly the philosophy and writing of behavioral objectives. Such information is readily available (see Bibliography) and should be consulted by those who are unfamiliar with behavioral objectives.

As stated earlier, preliminary behavioral objectives should be formulated immediately upon selection of the activity. In addition to serving an evaluative role, they are a source of guidance for planning the unit. However, they must not be allowed to limit innovative approaches to the study.

Once the activity is in progress, the preliminary objectives should be modified. If the activity is allowed to progress creatively, many unexpected, desirable goals will emerge. They must not be ignored because they were not recognized at the start. To the contrary, such goals should be incorporated into the list of objectives. It may be necessary to discard some of the preliminary behavioral objectives if they are found inappropriate. In this way the objectives evolve throughout the entire activity. As such they become representative of the activity rather than a list of idealistic goals developed for a file.

The revised behavioral objectives are used as guides in the formulation of an evaluative tool appropriate to the study. If properly constructed, the criteria and methods of evaluation will be stated within each objective.

In addition to fulfilling immediate planning and evaluative needs, the resulting behavioral objectives and data provide valuable tools for modifying the activity for future students.

References

Eiss, A., Behavioral Objectives in the Affective Domain, National Science Teachers Association, Washington, D. C., 1969. This useful guide for writing behavioral objectives includes specific examples encompassing psychomotor, cognitive and affective domains.

Koran, J., et al, How to Write Behavioral Objectives in Science Instruction, National Science Teachers Association, Washington, D. C., 1969. A discussion of the theory and expression of objectives concerned with affective behavior is presented.

Mager, R. F., Preparing Instructional Objectives, Fearon Publishers, Palo Alto, Calif., 1962. This is a programmed text for instruction in the writing of objectives.

Appendix 5

Bibliography

During this age of the "information explosion," it is imperative that students learn to use many appropriate sources while researching a particular problem. For this reason a listing of those books and pamphlets found particularly useful by teachers and students in the study of water pollution during the 1969 and 1970 summer programs and the 1969-1970 school year are included. It is hoped that most of the publications listed under Core References will be made available to students while studying water pollution. The references listed under Additional References are also excellent, but due to prior consideration on the technical level should probably be added only after the Core References have been secured.

This bibliography is by no means comprehensive. Hopefully, current periodicals, state and federal water surveys, for your area, and local library resources will supplement this reference listing.

Appendix 5

A. Core References

1. The following set of publications will provide a working reference source for any class, upper elementary through 12th grade. It is recommended that multiple copies of the asterisked publications be obtained, depending on class size and grade level. Those references with one asterisk (*) should be purchased in multiple copies for elementary through 8th grade use. The double asterisk (**) indicates that multiple copies could best be used for a 9th through 12th grade situation.

   a. **American Public Health Association, Standard Methods for the Examination of Water and Wastewater, (13th ed.), American Public Health Association, Inc., 1740 Broadway, New York, N. Y., 1971. This is an indispensible technical reference which includes test procedures and explanations of dissolved solids and gases found in water. The biology section includes collection methods and diagrams of organisms.

   b. Billings, W. D., Plants, Man, and the Ecosystem, (2nd ed.), Fundamentals of Botany Series, Wadsworth Publishing Co., Belmont, California, 1970. 160 pp. This paperback can introduce ecological concepts to 9th-12th graders and contains a section concerning man's effects on the environments.

   c. Carvajal, J., and M. E. Munzer, Conservation Education--A Selected Bibliography, The Interstate Printers and Publishers, Danville, Ill., 1968. 98 pp. This is an annotated bibliography dealing with not only water but also air, population, and land conservation.

   d. Frost, T. P., The Galloping Ghost of Eutrophy, Society for the Protection of New Hampshire Forests, 5 South State St., Concord, N. H., 1968. 36 pp. This is a well written pamphlet on the problem of eutrophication.

   e. Leopold, L. B., and K. S. Davis, Water, Life Science Library, Time Inc., New York City, 1966. 200 pp. This is a well illustrated book covering water use and hydrology (particularly useful for lower grade use).

f. **McKee, J. E., and H. W. Wolf, Water Quality Criteria, (2nd ed.), Water Quality Control Board, Sacramento, Calif., 1963. 548 pp. This is an excellent reference, although dated, on chemical, biological, radioactive, and pesticide pollutants. Each pollutant is explained and many toxicity levels are listed. 3827 references are listed.

g. **Needham, J. G., and P. R. Needham, A Guide to the Study of Fresh-Water Biology, Holden-Day, Inc., 500 Sansome St., San Francisco, Calif., 1969. 108 pp. This is an excellent reference book for any fresh-water biology work which is thoroughly illustrated with a good section on collection and equipment.

h. Ward, R. C., Principles of Hydrology, McGraw-Hill Book Co., New York City, 1967. Easily readable for high school level, it contains stimulating ideas for activities.

i. Environmental Education for Everyone--Bibliography of Curriculum Materials for Environmental Studies, National Science Teachers Association, 1201 Sixteenth St., N. W., Washington, D.C., 20036, 1970. 36 pp. The most comprehensive bibliography reviewed to date, it includes programs in environmental education curriculum guides, textbooks, experiments, enrichment readings, periodical listings, film strips, film lists, and other invaluable materials for environmental studies.

j. Simplified Laboratory Procedures for Wastewater Examination, WPCF Publication #18, Water Pollution Control Federation, 3900 Wisconsin Ave., Washington, D.C., 30016, 1969. 62 pp. Simplified Standard Method tests for physical and chemical examination of water and is best for use in grades 9-12.

2. The following publications may be purchased through the Superintendent of Documents, U.S. Government Printing Office, Washington, D. C. 20402.

   a. Austin, John H., A Primer on Waste Water Treatment, 1969. 24 pp., $.55. This is a clearly written pamphlet concerning the methods and problems of waste water treatment which is good for 7th-12th grade use.

b. *Baldwin, H. L., and C. L. McGuinnes, A Primer on Ground Water, 1966. 26 pp., $.25. This pamphlet explains ground water and ground-water resources competently for 7th-12th grade use.

c. *Leopold, L. B., and W. B. Langbein, A Primer on Water, 1966. 50 pp., $.35. This is a good introductory pamphlet on hydrology and water use, is good for 7th-12th grade use, and includes a glossary.

d. Ingram, W. M., K. M. MacKenthum and A. F. Bartsch, Biological Field Investigative Data for Water Pollution Surveys, 1966. 139 pp., $.70. This paperback has sections on graphical expression of data and organism response to organic pollution and has both good references and a glossary.

e. Thomas, H. E., The Yearbook of Agriculture, 1955: Water. 751 pp., $2.00. It contains numerous papers on water source and use and is an excellent book for the money... if it is still available from the Superintendent.

f. The Practice of Water Pollution Biology, 1969. 281 pp., $1.50. This paperback covers areas such as aquatic environments, organic wastes, toxic materials, acid mine and radioactive wastes, eutrophication, marine environments, water and waste treatment, and nuisance organisms and has a large reference section.

g. Water Quality Criteria, Federal Water Pollution Control Administration, 1968. 234 pp., $3.00. This book defines the various uses of water in the U.S. and recommends the various parameter limits for the different water uses. Good references and glossaries are included.

3. Individual copies of the following three pamphlets may be obtained free from Millipore Corporation, Bedford, Massachusetts, 01730.

   a. Experiments in Microbiology, 1969. 45 pp. An excellent pamphlet for 7th-12th grade use. Procedures and experiments to familiarize students with microorganisms--their occurrence in nature and ways to culture them--are given.

b. Microbiological Analysis of Water, 1969. 25 pp. Good procedures and explanations of coliform indicators and other bacteria are discussed.

c. Microbiology for the Beginning Student, 1969. 12 pp. Basic explanation of the membrane filtration technique and equipment is presented.

4. The following three pamphlets may be purchased for $.50 each through Educational Products Division, LaMotte Chemical Products Company, Chestertown, Md., 21620.

   a. *Amos, W. H., Limnology, 1969. 39 pp. This introduction to the fresh water environment is simply written and can be used by 7th-12th graders. Running and still water and the organisms found in each are discussed.

   b. Renn, C. E., A Study of Water Quality, 1968. 46 pp. This is a good pamphlet dealing with the general aspects of water pollution (usable in 7th-12th grade).

   c. Renn, C. E., Our Environment Battles Water Pollution, 1969. 32 pp. This is a good paperback, usable in elementary through 12th grade, dealing with various dissolved solids and gases and their relationship with organisms.

B. Additional References

The following list of references could be extremely beneficial in the study of water pollution. However, due to the price or technical nature, these texts should probably be purchased after the Core References have been obtained.

a. Chorley, R. J., (ed.), Water, Earth, and Man, Barnes and Noble, Inc., New York City, 1969. This is a good reference on hydrology and water use, capable of being used by 7th-12th graders.

b. Foerster, J. W., "A Phyco-periphyton Collector," Turtox News, 47-3, pp 82-84, 1969. It describes a simple, easy-to-make periphyton collector. The periphyton is collected on glass microscope slides.

c. McHarg, I. L., Design With Nature, Natural History Press, New York City, 1969. This is an excellent book which deals with the need for environmental planning as further development (of land) takes place (best for high school use).

d. Pelczar, M. J.,and R. D. Reid, Microbiology, McGraw-Hill Book Co., New York City, 1965. This is a microbiology text which includes introductions to the taxonomy, biochemistry, cultivation, control, and ecological role of microorganisms.

e. Pennak, R. W., Fresh-Water Invertebrates of the United States, Ronald Press Co., New York City, 1953. 769 pp. This is a thorough, technical text.

f. Sawyer, C. N.,and P. L. McCarty, Chemistry for Sanitary Engineers, (2nd ed.), McGraw-Hill Book Co., New York City, 1968. 518 pp. A thorough presentation of the theory and methods of sanitation chemistry is given. This book might best be used as a teacher's reference because a knowledge of elementary chemistry is assumed.

g. Smith, G. M., Fresh-Water Algae of the United States, McGraw-Hill Book Co., New York City, 1950. 719 pp. This is a comprehensive text on algae--collection, preservation, and methods of study.

h. Ward, H. B.,and G. C. Whipple, Fresh-Water Biology, (2nd ed.), John Wiley and Sons, New York City, 1959. This is the single most comprehensive manual for the identification of aquatic plants and animals.

i. Special Publication Number 1, Sources of Limnological and Oceanographic Apparatus and Supplies, American Society of Limnology and Oceanography. Many specialized items of biological collecting equipment are not available from the usual supply houses. This publication lists the suppliers. It is available from the Secretary of the Society.

C. Periodicals

To keep students aware of environmental news, a number of periodicals should be made available for classroom use. The following periodicals were found useful during the 1969-70 school year:

a. The Conservation Foundation Letter, (monthly), The Conservation Letter, 1717 Massachusetts Ave., N. W., Washington, D. C. 20036, $6/yr. This is a three-hole punched newsletter capable of being accumulated for reference in a loose-leaf notebook and deals with current legislation and all types of environmental news. It is probably best for high school use.

b. Environmental Science and Technology, (monthly), American Chemical Society, 20th and Northampton Sts., Easton, Pa. 18042, $7/yr. This is a technical magazine but is good for high school use.

c. Environment, (10 issues/yr.), Environment, Circulation Dept., 438 North Skinker Boulevard, St. Louis, Mo. 63130, $8.50/yr. This is a well written periodical, dealing in depth with current environmental problems (capable of use in grades 7th-12th).

D. Movies

During the 1969-1970 school year, Miss Elizabeth Gage and her students at Northfield School reviewed the following environmental films. The film reviews worked on during the 1970 summer programs at Tilton have not yet been compiled but will be forthcoming in the newsletters.

Title: It's Your Decision: Clean Water

Source: Association Films, Inc.

Regional Film Centers:

600 Grand Avenue, Ridgefield, N. J. 07657

561 Hillgrove Avenue, LaGrange, Ill. 60525

324 Delaware Avenue, Allegheny County, Oakmont, Pa. 15139

Information: Color, 14 minutes, free loan.

Summary: The movie begins by briefly tracing the development of towns and cities along rivers and streams. It shows how rivers were able to handle their pollutional load, but with our rapidly growing population, the pollution crisis has become more acute. Animated figures are used to show the basic characteristics of polluted water and how primary and secondary water treatment plants operate.

Appraisal: Because the movie is easy to understand, it could be shown to several different types of audiences. Clean Water would be effective as an introductory film for a pollution course. The film would be of benefit to elementary school students if seen as an introduction to our nation's pollution problems. The film would also be very effective if shown in a community where a secondary water treatment plant is needed. It could be shown to the general public, too, possibly as a short feature in a movie theatre.

Title: Oops, or How Broad Shoulders Polluted the River

Source: University of Minnesota, Audio-Visual Department, 2037 University Avenue, S. E., Minneapolis, Minn. 55455.

Information: Black and white (Color: $5.85), 22 minutes.

Summary: This movie gives insight into industry's problems in controlling pollution, listing several different aspects such as analyzing wastes, repairing equipment, showing concern for waste treatment plants, and spreading responsibility to everyone. It stresses the importance on competent, well-trained employees. The title refers to a worker, "broad shoulders," who through inattentiveness, poor management, and poor training created a big mess of overflowing tanks of chemicals, petroleum, and suds.

Appraisal: The class felt that this movie was good for our purpose but is best suited for its intended purpose of making industry aware of the problems of pollution and suggesting how these problems can be solved. It was good for our class because we have little opportunity to come into contact with industry, their problems, and their attempts to remedy the situations.

Title: Becket Adventures

Source: Sid Dupont, Becket Academy, East Haddam, Conn.

Information: Color, 30 minutes.

Summary: Mr. Dupont visits schools showing films or slides of the canoe trip that Becket summer students take down the Connecticut River from its source to the sound. The boys on the trip surveyed points along the river, recording animal and plant life in the area, bottom structure, flow, weather, geology and the condition of the river itself. This information was written in a log which may be obtained from Becket Academy.

Appraisal: Although this film was made by amateurs, we feel it is useful for obtaining a general picture of the condition of the Connecticut. We feel that Mr. Dupont wants others to become aware of the environment in which they live through this movie, just as the boys became aware through their trip. We feel that this is a worthwhile movie for students of pollution to see.

Title: Story of a Lake

Source: Chevron Chemical Company, Advertising and Public Relations, Ortho Division, 200 Bush Street, San Francisco, Calif.

Information: Color, 10-15 minutes, free loan (one day).

Summary: The film describes the conditions in a small lake choked with water weed (Elodea) and includes interviews of homeowners who describe the effect of the lake in this condition on their recreational enjoyment of the lake and on property values of lake sites. The people try to control the growth of water weed by raking it out but find that this offers only temporary control. The lake community calls on the services of a professional herbicide company which sprays the lake with the chemical, Diquat, a product of the Chevron Company. There are scenes showing the lake being sprayed from a small boat. The effects of the chemical are described in rather general terms ("...attacks green matter and breaks down cell structure..."). The herbicide takes effect in 4-5 days and after 10-14 days the water weed has decomposed. Views of the clean lake are shown and community members are interviewed for their impressions.

Appraisal: The film is clearly selling Diquat, a product of the Chevron Chemical Company, and the biological content of the film is minimal. It is, in our opinion, not as useful for water pollution studies as it might be. One wonders if the herbicide is "harmless to water animals," why the men spraying the water are wearing coveralls and masks while they are working. Further, we wonder specifically what effect the chemical has on cells...maybe a letter to the Chevron Company would clear this up for us.

Appendix 5

Title: Membrane Microfiltration: A New Tool for Classroom Science

Source: Bernard I. Sohn, Educational Division, Millipore Corporation,
Bedford, Mass. 01730.
Also, Tilton School has this film to lend upon request.

Information: Color, 30 minutes, free loan.

Summary: The movie dealt specfically with Millipore apparatus, its uses in the classroom, in the field, in industry, and in medicine. It also stressed the advantage of the Millipore over previous techniques of filtering by using clever photography. Animated figures representing bacteria described the filtering process and its usefulness in detecting the presence of micro-organisms.

Appraisal: Although this film was intended for showing to teachers, we feel it should be used as an introduction for students as well. The film is simple yet comprehensive in its coverage of the subject.

Title: Water: Pattern of Life

Source: Ohio Department of Natural Resources, Administrative
Services Section, 1500 Dublin Road, Columbus,
Ohio 43212.

Information: Color, free loan.

Summary: This movie told about the sources of, uses of, and problems with natural water in the state of Ohio. It covered many topics such as the hydrologic cycle, transportation, droughts, flood control, underground water supply, growing population, recreational uses of water, industrial uses of water, reservoirs, and quality control of water. It offered suggestions as how to solve problems; for example, surveying, control of flood plains, and long range planning of water use and supply. It also suggested that areas should be divided into watershed basins to control the problems.

Appraisal: This film would be good as an introduction to water pollution or biology because it shows the wide usage of water. It does not, however, deal specifically with pollution or control of it. It also is somewhat outdated because it speaks of Lake Erie as a valuable source of water, which it was at one time!!

Appendix 5

Title: What Are We Doing to Our World (in two parts)

Source: University of Minnesota, Audio-Visual Department, 2037
University Avenue, S. E., Minneapolis, Minn. 55455.

Information: Color, 25 minutes each, $7.20 each reel.

Summary: "We are going to have to choose between the advancements of technology. . ." and the necessity of maintaining ecological balances on earth. This film explores some serious consequences of unrestricted growth, pollution, waste, and over-population problems.

What was a question of conservation on earth is becoming a question of survival. Experiments such as the one at Hubbard Brook attempt to understand ecological balances and explore some of the unforeseen consequences of man's attempts to make a better world.

Appraisal: This is an excellent film which provokes very good discussion among students. It is an unbalanced film from the point of view that it stresses what we are doing to pollute and far less is said about what we might do to prevent pollution. In several places, it is brought out that people will have to re-examine their attitudes and values concerning their relation to the earth environment and this serves to balance the views presented. The spokesman for the Agricultural Chemists presents some interesting arguments about continued use of pesticides.

Title: Municipal Sewage Treatment Processes

Source: Communicable Diseases Center, Atlanta, Ga. 30333.

Information: Black and White, 13 minutes, free loan.

Summary: This movie begins with a definition of municipal sewage, where it goes, and the results of its discharge. It goes into great detail with "on location" scenes about the different types of sewage treatment plants. The movie then gives a basic outline of the steps a city or town must follow in planning the design, construction, and maintenance of a plant. A simple basis for choosing the most effective type of treatment is given and summarizes the results that people can expect from their plant. The movie also goes into some detail about the operation of water treatment plants.

Appraisal: Since the movie is clear and easily understood in its description of sewage and its treatment processes it could be an effective way to inform the people of a town of the need for a treatment plant and of the decisions they will have to make. Though the movie is somewhat dated, it could be used early in a water pollution course.

Title: The River Must Live

Source: Shell Film Library, 450 North Meridian Street,
Indianapolis, Ind. 46204.

Information: Color, 21 minutes, free loan.

Summary: This film deals with the growth of industry as a cause of the growth in pollution. The fact that one person's effluent is another's supply is mentioned. Processes of decay of life in the river through bacteria and the different forms of life contained within an ecosystem are discussed. The film speaks of how the pollutional imbalance decreases the supply of oxygen. There is a need for time in order to restore the natural balance. When there is too much pollution the river dies. After being dumped in the river, the pollution is carried to and lost in the sea's vastness. From there, through the hydrocycle, the water is purified and returned to the land. Different methods of waste treatment can purify the water before it reaches the sea. The film also treats the different uses of water--industrial, commercial, and domestic.

Appraisal: This film was rated very good to excellent. The narration was easy to follow, the organization was good, and the music was appropriate. There was excellent visual treatment of uses of water and different types of waste water treatment. Also, the Shell Company refrained admirably from pushing its product. They did skim over the possibility of oceanic pollution, ignoring the tertiary treatment factor. The best use of this film would be as an introduction in water pollution.

E. Equipment

Various companies are selling commercial water testing equipment suitable for classroom use. Catalogs and descriptive literature can be obtained from the following addresses.

1. Chemical testing equipment

    a. Hach Chemical Company
    Box 907
    Ames, Ia. 50010

b. Delta Scientific Corporation
120 East Hoffman Ave.
Lindenhurst, N. Y. 11757

c. LaMotte Chemical Products Company
Educational Products Division
Chestertown, Md. 21620

2. Bacteriological equipment

Millipore Corporation
Educational Products Division
Bedford, Mass. 01730

3. Aquatic Biology Equipment

a. Oceanography Unlimited, Inc.
108 Main St.
Lodi, N. J. 07644

b. Wildlife Supply Company
2200 S. Hamilton St.
Saginaw, Mich. 48602

Appendix 6

Water Pollution and Environmental Glossary

This glossary is a compilation of terms from aquatic ecological, hydrologic and chemical fields of endeavor. Of the many persons who contributed, the principle contributor was John E. Mathews of the Department of the Interior (Robert S. Kerr Water Research Center, Ada, Oklahoma).

Terms underscored in a definition are separately defined in this Glossary. When appropriate, closely associated or related terms are cited parenthetically, following the definition. Specific synonyms are noted in parentheses with the listed word.

Appendix 6

A

ABIOTIC FACTOR Physical, meteorological, geological, or chemical aspect of environment.

ABYSSAL ZONE All of a sea or of a very deep lake below the bathyal zone. The primary energy source for this region lies far above in the euphotic zone; density of life depends on the amount of organic material that settles from the euphotic zone. (See Hadal zone.)

ACCLIMATION Physiological and behavioral adjustments of an organism in response to a change in environment. (See Adaptation.)

ACCLIMATIZATION Acclimation of a particular species over several generations in response to marked environmental changes.

ACID A hydrogen ion (H^+) donor.

ACIDITY See Appendix 1: Chemistry.

ACTINOMYCETES Filamentous microorganisms intermediate between the fungi and bacteria, although more closely related to the bacteria. These organisms are widely distributed in soils and are often conspicuous in lake and river muds. They are often associated with taste and odor problems in water supplies.

ACUTE TOXICITY Any toxic effect that is produced within a short period of time, usually 24-96 hours. Although the effect most frequently considered is mortality, the end result of acute toxicity is not necessarily death. Any harmful biological effect may be the result. (See Chronic Toxicity, Direct Toxicity.)

ADAPTATION Change in the structure, form or habits of an organism to be better fit changed or existing environmental conditions. (See Acclimation.)

AEROBIC Refers to life or processes occuring only in the presence of free oxygen; refers to a condition characterized by an excess of free oxygen in the aquatic environment. (See Anaerobic.)

ALGAE (Alga) Simple plants, many microscopic, containing chlorophyll. Algae form the base of the food chain in aquatic environments. Some species may create a nuisance when environmental conditions are suitable for prolific growth.

ALKALINITY Appendix 1: Chemistry.

ALLOCHTHONOUS Pertaining to those substances, materials, or organisms in a waterway which originate outside and are brought into the waterway. (See Autochthonous.)

ALLUVIAL FAN (Delta)

ANABOLISM Synthesis or manufacture of organic compounds within an organism. (See Metabolism.)

ANADROMOUS Pertaining to fishes that spend most of their life in salt water but enter freshwater to spawn (e.g., salmon, shad, striped bass, etc.). (See Catadromous.)

ANAEROBIC Refers to life or processes occurring in the absence of free oxygen; refers to conditions characterized by the absence of free oxygen. (See Aerobic.)

ANTAGONISM Reduction of the effect of one substance because of the introduction or presence of another substance (e.g., one substance may hinder, or counteract, the toxic influence of another). (See Synergism.)

APHOTIC ZONE That portion of a body of water to which light does not penetrate with sufficient intensity to have any biological significance. (See Euphotic Zone.)

ARTIFICIAL SUBSTRATE A device placed in the water (for a specified period of time) that provides living spaces for a multiplicity of organisms (e.g., glass slides, concrete blocks, multiplate samplers, rock baskets, etc.). The primary purpose of artificial substrates is to allow the investigator to collect organisms in areas where the physical habitat is limiting or cannot be adequately sampled using conventional methods.

ASSIMILATION 1. Removal of dissolved or suspended materials from a water mass by biological, chemical, and physical processes; 2. Conversion or incorporation of absorbed nutrients into body substances. (See Synthesis.)

ASSOCIATION All organisms occupying a given habitat.

ATOLL Large, thick, coral mass encircling a lagoon in tropical oceans; sometimes portions of the reef become built up with sand, silt, soil and vegetation to become an island. (See Barrier Reef, Fringing Reef.)

AUFWUCHS (Periphyton)

AUTOCHTHONOUS Pertaining to those substances, materials, or organisms originating within a particular waterway and remaining in that waterway. (See Allochthonous.)

AUTOTROPHIC (Holophytic) Self nourishing; denoting those organisms that do not require an external source of organic material but can utilize light energy and manufacture their own food from inorganic materials (e.g., green plants, pigmented flagellates). (See Heterotrophic.)

B

BARRIER BEACH A ridge of deposits separated from the mainland by an interval of water.

BARRIER REEF Large, thick, coral mass more or less surrounding an island or paralleling the mainland shore in tropical areas and separated from the land mass by a lagoon. (See Atoll, Fringing Reef.)

BASE A hydrogen ion (H^+) acceptor.

BATHYAL ZONE That region of the sea that extends from the euphotic zone to the bottom of the continental slope. Density of life in this zone depends on organic material settling from the euphotic zone and is generally inversely proportional to the depth.

BEACH The zone of demarcation between land and water of lakes, seas, etc., covered by sand, gravel or larger rock fragments.

BENTHIC REGION The bottom of a waterway; the substratum that supports the benthos.

BENTHOS Bottom-dwelling organisms. These include: (1) sessile animals such as sponges, barnacles, mussels, oysters, worms, and attached algae; (2) creeping forms such as snails, worms and insects; (3) burrowing forms, which include clams, worms, and some insects; and (4) fish whose habits are more closely associated with the benthic region than other zones (e.g., flounders).

BIOASSAY A determination of the biological effect of some substance, factor or condition employing living organisms or cells as the indicator.

BIOCHEMICAL OXYGEN DEMAND See Appendix 1: Chemistry.

BIOCOENOSIS The plants and animals comprising a community.

BIOLOGICAL CONTROL 1. Use of natural predators, parasites, or pathogens to reduce or eliminate pest organisms (e.g., use of gambusia to feed on mosquito larvae). 2. Control of organisms by interference with their physiological processes (e.g., sterilization of male flies).

BIOMASS The total amount of living material in a particular habitat or area; an expression dealing with the total weight of a given population of organisms.

BIOMONITORING 1. Continuous surveillance of an effluent (or dilution thereof) by using living organisms to test its suitability for discharge into a receiving water. 2. Use of living organisms to test the quality of a receiving water downstream from a waste discharge. (See Bioassay.)

BIOTA All life of a region.

BIOTIC FACTORS (Biological Factors) In ecology, those environmental factors which are the result of living organisms and their activities; distinct from physical and chemical factors (e.g., competition, predation, etc.). (See Ecological Factor.)

BIOTIC POTENTIAL The inherent capability of an animal to multiply in an unrestricted environment. (See Environmental Resistance.)

BIOTOPE (Habitat)

BLOODWORMS Midge fly larvae. Many of the species have hemoglobin in the blood causing a red color and are often associated with rich organic deposits. Also, the common name for certain of the marine segmented worms (class Polychaeta). (See Sludgeworms.)

BLOOM A readily visible, concentrated growth or aggregation of minute organisms, usually algae, in bodies of water.

BRACKISH WATERS Those areas where there is a mixture of fresh and salt water; the salt content is greater than fresh water but less than sea water; the salt content is greater than in sea water.

BUFFER SOLUTION A solution which, within limits, resists changes in pH.

C

CARNIVOROUS Pertaining to animals that feed on other animals. (See Herbivorous.)

CARRYING CAPACITY The maximum quantity of organisms that any particular habitat can support over an extended period.

CATABOLISM The breakdown of organic compounds within an organism. (See Metabolism.)

CATADROMOUS Pertaining to fish that spend most of their life in freshwaters but migrate to the sea to spawn (e.g., american eel). (See Anadromous.)

CATASTROPHIC DRIFT Massive drift of bottom organisms under conditions of stress such as floods or toxicity. (See Drift Organisms, Incidental Drift, Periodic Drift.)

CHEMICAL STRATIFICATION A layering of water in a lake because of density differences owing to the varying or differential concentrations of dissolved substances with depth. (See Stratification.)

CHLOROPHYLL Green photosynthetic pigment present in many plant and some bacterial cells. There are seven known types of chlorophyll; their presence and abundance vary from one group of photosynthetic organisms to another.

CHRONIC TOXICITY Toxicity, marked by a long duration, that produces an adverse effect on organisms. The end result of chronic toxicity can be death although the usual effects are sublethal (e.g., inhibits reproduction, reduces growth, etc.). These effects are reflected by changes in the productivity and population structure of the community. (See Acute Toxicity.)

CLASSIFICATION The placing of organisms into taxa (or categories) according to established scientific requirements. (See Taxonomy, Taxon.)

CLEAN WATER ASSOCATION An association of organisms found in any natural, unpolluted environment. These associations are characterized by the presence of species that are sensitive to environmental changes caused by introduction of pollutants. In many cases the presence of a wide variety of species with relatively few individuals representing any one of them is also characteristic. (See Sensitive Organisms, Tolerant Association.)

CLINOMETER The standard instrument used by geographers to measure the slope of a hillside.

COASTAL PLAIN A plain between the sea and higher land, usually at a low elevation.

COASTAL WATERS Those waters surrounding the continent which exert a measurable influence on uses of the land and on its ecology. The Great Lakes and the waters to the edge of the continental shelf.

COASTAL ZONE Coastal waters and adjacent lands which exert a measurable influence on the uses of the sea and its ecology. The zone extends onshore to the upper reaches of the tidal zone and adjacent shore areas. (See Estuary.)

COLD-BLOODED ANIMALS Animals that lack an internal temperature regulating mechanism to offset external temperature changes. Their body temperature fluctuates to a large degree with that of their environment. Examples are fish and aquatic invertebrates.

COLONY A distinguishable localized population within a species.

COMMUNITY All forms of life inhabiting a common environment.

COMPENSATION LEVEL The depth of a waterway at which there is a balance between photosynthesis and respiration.

COMPETITION The effort of two or more individuals or species of a community to utilize some of the same environmental resources.

COMPETITION EXCLUSION PRINCIPLE (Gause's Rule) No two species can occupy the same niche at the same time.

CONSUMERS Organisms which feed upon other organisms; often divided into first order consumers (Herbivores), second order (or higher) consumers (Carnivores which eat primary consumers), etc. (See Heterotrophic, Trophic Level.)

CONTINENTAL SHELF The shallow, gently sloping portion of the sea bottom bordering a continent, down to a depth of about 200 meters.

CONTINENTAL SLOPE The steeply sloping portion of the sea bottom extending seaward from the continental shelf.

CORAL A marine member of the phylum Coelenterata which secretes a hard exoskeleton, chiefly of calcium carbonate.

CORAL REEF Large coral mass associated with coastal areas in the tropics. (See Barrier Reef, Fringing Reef, Atoll.)

CRITERIA (Water Quality Criteria)

CRITICAL LEVEL (Threshold)

CRITICAL RANGE In bioassays, the value range of any factor between the maximum level or concentration at which no organisms die to the minimum level or concentration at which all organisms die under a given set of conditions in a given period of time.

CULTURAL EUTROPHICATION Acceleration by man of the natural process of enrichment (aging) of bodies of water.

CULTURE Cultivation of organisms in a medium containing necessary nutrients.

D

DECOMPOSERS (Reducers)

DELTA (Alluvial Fan) A fan-shaped deposition of silt, sand, gravel or other materials from a stream which occur when the hydraulic gradient lessens abruptly, as in the discharge of a stream into a lake or of a river into an ocean.

DENSITY (Population, Species) The number of individuals in relation to the space in which they occur; refers to the closeness of individuals to one another.

DENSITY STRATIFICATION (Stratification)

DEPOSITING SUBSTRATES Bottom areas where solids are being actively deposited. These often occur in the vicinity of effluent discharges. (See Sludge Deposits.)

DETRITUS Fragments of detached or broken down material.

DIFFUSION The even mixing of one compound throughout another.

DIRECT TOXICITY Toxicity that has an effect on organisms themselves instead of having an effect by actual alteration of their habitat or interference with their food supply. (See Acute Toxicity, Chronic Toxicity, Indirect Toxicity.)

DISSOCIATION The separation of preexisting ions during the process of solution.

DISSOLVED OXYGEN See Appendix 1: Chemistry.

DISSOLVED SOLID Any substance which existed primarily as a solid prior to the solution process.

DIURNAL 1. Refers to an event, process, or specific change that occurs every day; usually associated with changes from day to night. 2. Pertaining to those organisms that are active during day time. (See Nocturnal.)

DIVERSITY Pertaining to the variety of species within a given association of organisms. Areas of high diversity are characterized by a great variety of species; usually relatively few individuals represent any one species. Areas with low diversity are characterized by a few species; often relatively large numbers of individuals represent each species.

DOMINANT Species which by their activity, behavior, or number, have considerable influence or control upon the conditions of existence of associated species; species which "controls" its habitat and food web. (See Predominant.)

DRIFT ORGANISMS Benthic organisms temporarily suspended in the water and carried downstream by the current. (See Incidental Drift, Periodic Drift, Catastrophic Drift.)

DYSTROPHIC LAKES Shallow lakes with brown water, high organic matter content, low nutrient availability, poor bottom fauna, and high oxygen demand; oxygen is continually depleted and pH is usually low. In lake aging the "age" between a eutrophic lake and a swamp.

E

EBB TIDE That period of tide between a high water and the succeeding low water; falling tide. (See Flood Tide.)

ECOLOGICAL FACTOR Any part or condition of the environment that influences the life of one or more organisms. (See Biotic Factor.)

ECOLOGICAL NICHE The role of an organism in the environment, its activities and relationships to the living and nonliving environment; food and nutrition relationships are of primary importance. (See Habitat Niche.)

ECOLOGY Interrelationships between organisms and their environment, abiotic and biotic.

ECOSYSTEM A community, including all the component organisms, together with the environment, forming an interacting system.

ECOTYPE (Habitat Form) The growth form or appearance of an organism which is characteristic of a specific habitat. (Individuals of the same species may appear different in various habitats.)

EMERSED (Emergent) AQUATIC PLANTS Plants that are rooted at the bottom of a body of water, but project above the surface (e.g., cattails, bulrushes, etc.). (See Floating Aquatic Plants, Submersed Aquatic Plants.)

END-POINT The point at which a titration is to be terminated, sometimes signifying the presence of equivalent amounts of reactants.

ENRICHMENT An increase in the quantity of nutrients available to aquatic organisms for their growth and development. (See Eutrophication.)

ENVIRONMENT All external influences and conditions affecting the life and development of an organism.

ENVIRONMENTAL RESISTANCE Restriction imposed on the numerical increase of a population by environmental factors. (See Biotic Potential.)

EPILIMNION The water mass extending from the surface to the thermocline in a stratified body of water; the epilimnion is less dense than the lower waters and is wind-circulated and essentially homothermous. (See Hypolimnion.)

EQUILIBRIUM 1. The condition in which a population or community is maintained with only minor fluctuations in composition over an extended period of time. Sometimes called Dynamic equilibrium. 2. A dynamic interaction of two opposing chemical or physical processes occurring at equal rates.

ESTUARY That portion of a coastal stream influenced by the tide of the body of water into which it flows; a bay, at the mouth of a river, where the tide meets the river current; an area where fresh and marine waters mix. (See Positive Estuary, Inverse Estuary, Neutral Estuary, Coastal Zone.)

EULITTORAL ZONE (Tidal Zone)

EUPHOTIC ZONE The lighted region of a body of water that extends vertically from the water surface to the depth at which photosynthesis fails to occur because of insufficient light penetration.

EURY- Prefix meaning wide (e.g., euryhaline refers to a wide range of salinity tolerance; eurythermal refers to a wide range of temperature tolerance). (See Steno-.)

EUTROPHIC LAKES Lakes which are rich in nutrients and organic materials, therefore, highly productive. These lakes are often shallow and seasonally deficient of oxygen in the hypolimnion. (See Oligotrophic Lakes.)

EUTROPHICATION The natural process of the maturing (aging) of a lake; the process of enrichment with nutrients, especially nitrogen and phosphorus, leading to increased production of organic matter. (See Cultural Eutrophication, Oligotrophic Lakes, Eutrophic Lakes.)

EVAPOTRANSPIRATION The total of the transpiration of the plants of an area plus the evaporation of water from the area equals the total loss in the form of vapor.

F

FALCULTATIVE Refers to the capability of an organism to live under varying conditions (e.g., a falcultative anaerobe is an organism that although usually living in the presence of free oxygen can live in the absence of free oxygen). (See Obligate.)

FALL OVERTURN A physical phenomenon that may take place in a body of water during early autumn. The sequence of events leading to fall overturn include: (1) cooling of surface waters, (2) density change in surface waters producing convection currents from top to bottom, (3) circulation of the total water volume by wind action, and (4) vertical temperature equality. The overturn results in a uniformity of the physical and chemical properties of the entire water mass. (See Spring Overturn.)

FATHOM A unit of measurement equal to 6 feet (1.83 meters).

FAUNA Animal life.

FECAL COLIFORM See Appendix 1: Bacteriology.

FECAL STREPTOCOCCUS See Appendix 1: Bacteriology.

FIRTH A narrow arm of the sea; also the opening of a river into the sea. (See Estuary.)

FJORD (Fiord) A narrow arm of the sea between highlands. (See Firth, Estuary.)

FLOATING AQUATIC PLANTS Rooted plants that wholly or in part float on the surface of the water (e.g., water lillies, water hyacinth and duckweed). (See Emersed Aquatic Plants, Submersed Aquatic Plants.)

FLOOD TIDE That period of tide between low water and the succeeding high water; a rising tide. (See Ebb Tide.)

FLORA Plant life.

FOOD CHAIN Dependence of a series of organisms, one upon the other, for food. The chain begins with plants and ends with the largest carnivores (e.g., phytoplankton, zooplankton, forage fish, game fish). Food chains usually do not exist in nature; they are parts of food webs.

FOOD CYCLE (Food Web) All the interconnecting food chains in a community.

FORAGE FISH Fish, usually smaller species, that are important as food for other species.

FREE-SWIMMING (Motile) Actively moving about in water or capable of moving about in water. (See Sessile.)

FRINGING REEF Large coral mass at the edge of any land mass in tropical seas; it begins at the water's edge and may extend out to a quarter mile. (See Barrier Reef, Atoll.)

G

GAME FISH (Sport Fish) Those species of fish considered to possess sporting qualities on fishing tackle (e.g., salmon, trout, black bass, striped bass, etc.). Game fish are usually more sensitive to environmental changes than rough fish.

GAUSE'S RULE (Competition-Exclusion Principle)

GROUND WATER The body of water derived from percolation; contained in the soil, sub-soil and underlying rocks of an area.

H

HABITAT (Biotype) A specific type of place that is occupied by an organism, a population, or a community.

HABITAT FORM (Ecotype)

HABITAT NICHE The specific part or smallest unit of a habitat occupied by an organism. (See Ecological Niche.)

HADAL ZONE Pertaining to that part of the ocean at depths exceeding 6,000 meters, including both water and floor or bottom. (See Abyssal Zone.)

HERBIVORE An organism that feeds on plant material; a first order consumer. (See Carnivore.)

HETEROGENEOUS Consisting of dissimilar elements or constituents. (See Homogeneous.)

HETEROTROPHIC (Holozoic) Pertaining to organisms that are dependent on organic material for food. (See Autotrophic.)

HIGHER AQUATIC PLANTS (Pond Weeds) Those plants whose seeds germinate in the water phase or substrate of a body of water and which must spend part of their life cycle in water. This grouping includes plants which grow completely submersed as well as a variety of emersed and floating leaf types.

HOLOPHYTIC (Autotrophic)

HOLOZOIC (Heterotrophic)

HOMOGENEOUS Of uniform composition throughout.

HOMOTHERMOUS Having the same temperature throughout.

HYDROLYSIS The reaction of a salt with water to produce a basic or acidic solution.

HYPOLIMNION The region of a body of water that extends from the thermocline to the bottom and is essentially removed from major surface influences. (See Epilimnion.)

I

IDENTIFICATION The use of a taxonomic key or the equivalent to determine the scientific name of an organism.

INCIDENTAL DRIFT The casual, random drift of organisms. (See Drift Organisms, Catastrophic Drift, Periodic Drift.)

INDICATOR 1. A substance which, by means of a color change, identifies the end-point of a titration. 2. A substance which, by means of a color change, qualitatively and/or quantitatively evaluates the presence of an unknown substance.

INDIRECT TOXICITY Toxicity that affects organisms by interfering with their food supply or modifying their habitat instead of directly acting on the organisms themselves. (See Direct Toxicity.)

INFILTRATION The term used by hydrologists to describe the gradual downward flow of water from the surface through soil to ground water and water table reservoirs.

INLET A short, narrow waterway connecting a bay, lagoon, or similar body of water with a large parent body of water; a stream which flows into a lake; an arm of the sea, or other body of water, that is long compared to its width and that may extend a considerable distance inland.

INSTAR A stage in the life cycle of an insect or other arthropod between two successive molts.

INTERACTION Mutual or reciprocal action or influence between organisms, between organisms and environment, or between environmental factors.

INTERSPECIFIC Refers to relations or conditions between species. (See Intraspecific.)

INTERTIDAL ZONE (Tidal Zone)

INTOLERANT ORGANISMS (Sensitive Organisms)

INTRASPECIFIC Refers to relations or conditions between individuals within a species. (See Interspecific.)

INVERSE ESTUARY Type of estuary in which evaporation exceeds the supply of freshwater; evaporation freshwater inflow + precipitation. (See Positive Estuary, Neutral Estuary.)

INVERTEBRATES. Animals without an internal skeletal structure (e.g., insects, mollusks, crayfish). (See Vertebrate.)

ION An atom or group of atoms which has become charged either by loss or by gain of one or more electrons.

L

LAGOON 1. A shallow sound, pond, or channel near or communicating with a larger body of water. 2. A settling pond for treatment of wastewater.

LARVA The immature form of an animal which is unlike its parents. Larvae are usually self-feeding but must pass through some sort of metamorphosis before assuming the characteristics of the adult; in insects, the wormlike stage between the egg and the pupa.

LAW OF THE MINIMUM, LIEBIG'S "The growth and reproduction of an organism is dependent on the nutrient substance, such as oxygen, carbon dioxide, calcium, etc., that is available in minimum quantity." (See Limiting Factor.)

LAW OF TOLERANCE, SHELFORD'S "When one environmental factor or condition is near the limits of toleration, either minimum or maximum, that one factor or condition will be the controlling one and will determine whether or not a species will be able to maintain itself." (See Limiting Factor.)

LEACHING The process by which nutrients in the soil are dissolved and carried away by water flowing through it by processes such as percolation.

LENTIC Pertaining to standing (nonflowing) waters such as lakes, ponds, and swamps. (See Lotic.)

LIFE CYCLE The various phases, changes, or stages through which an individual passes from the fertilized egg to death of the mature organism. Briefly stated it is birth-maturation-reproduction-death.

LIMITING FACTOR A factor whose absence, or excessive concentration, exerts some restraining influence upon a population through incompatibility with species requirements or tolerance. (See Law of the Minimum, Law of Tolerance.)

LIMNETIC ZONE The open-water region of a lake, especially in areas too deep to support rooted aquatic plants. This region supports plankton and fish as the principal plants and animals. (See Littoral Zone.)

LIMNOLOGY The ecology of fresh waters.

LITTORAL ZONE The shallow area that extends from shore to the lakeward limit of rooted aquatic plants; the shoreward region of a body of water; in marine ecology, the tidal zone. (See Limnetic Zone.)

LOTIC Pertaining to flowing waters such as streams and rivers. (See Lentic.)

M

MACROORGANISMS Those organisms retained on a U.S. standard sieve No. 30 (openings of 0.589 mm); those organisms visible to the unaided eye. (See Microorganisms.)

MACROPHYTE Any plant that can be seen with the naked, unaided eye (e.g., aquatic mosses, ferns, liverworts, rooted plants, etc.).

MEDIAN TOLERANCE LIMIT (TL_m) The concentration of tested substance in water at which just 50% of the test organisms survive for a specified period of exposure. (See Tolerance Limit.)

MEROMICTIC LAKES Lakes in which dissolved substances create a gradient of density differences with depth; this prevents complete mixing or circulation of water masses. (See Chemical Stratification.)

MEROMIXIS A condition of permanent stratification of water masses in lakes.

MESOLIMNION (Thermocline)

METABOLISM The sum of all chemical processes occuring within an organism; includes both synthesis (anabolism) and breakdown (catabolism) of organic compounds.

METALIMNION (Thermocline)

METAMORPHOSIS Distinct transformation of an animal from one distinctive life history stage to another in its postembryonic development (e.g., larva of an insect to a pupa). (See Life Cycle.)

MICROORGANISMS Those organisms retained on a U.S. standard sieve No. 100 (openings of 0.149mm); those minute organisms invisible or only barely visible to the unaided eye. (See Macroorganisms.)

MOLARITY A concentration unit which denotes the number of moles of particles (molecules or ions) present in 1 liter of solution.

MOLE A collective unit which signifies 6×10^{23} of anything but is used primarily when dealing with molecules or ions.

MOLT To cast or shed periodically the outer body covering which permits an increase in size. This is especially characteristic of invertebrates. (See Instar.)

MOTILE (Free swimming)

N

NANOPLANKTON Very minute plankton not retained in a plankton net equipped with No. 25 silk bolting cloth (mesh, 0.03 to 0.04 mm).

NATURAL SELECTION Processes occurring in nature which result in selective survival and elimination of individuals less well adapted to their environment.

NAUPLIUS Free-swimming microscopic larval stage characteristic of many crustaceans, barnacles, etc.

NEAP TIDES Exceptionally low tides which occur twice each month when the earth, sun and moon are at right angles to each other; these usually occur during the moon's first and third quarters. (See Spring Tides.)

NEKTON Macroscopic organisms swimming actively in water (e.g., fish). (See Plankton.)

NERITIC ZONE Relatively shallow water zone which extends from the high-tide mark to the edge of the continental shelf.

NET PLANKTON Plankton retained in a plankton net equipped with No. 25 silk bolting cloth (mesh, 0.03 to 0.04 mm).

NEUSTON Organisms associated with, or dependent upon, the surface film (air-water interface) of bodies of water.

NEUTRAL ESTUARY Type of estuary in which neither the freshwater inflow nor the evaporation predominates; freshwater inflow + precipitation = evaporation. (See Positive Estuary, Inverse Estuary.)

NEUTRALIZATION The process of nullifying the effects of an acid (base) through the addition of a base (acid), usually accompanied by the formation of salt, water, and heat.

NICHE See Ecological Niche, Habitat Niche.

NOCTURNAL Pertaining to those organisms that are active at night. (See Diurnal.)

NUISANCE ORGANISMS (Pests) Those organisms capable of interfering with the use or treatment of water.

NUTRIENTS 1. Elements, or compounds, essential as raw materials for organism growth and development (e.g., carbon, oxygen, nitrogen, phosphorus, etc.). 2. The dissolved solids and gasses of the water of an area.

NYMPH An immature developmental form characteristic of the pre-adult stage in insects that do not have a pupal stage (e.g., May flies and stone flies). (See Larva.)

O

OBLIGATE Limited to one mode of life or action. (See Facultative.)

OCEANIC ZONE The region of open ocean beyond the continental shelf.

OLIGOTROPHIC LAKES Deep lakes which have a low supply of nutrients, thus they support very little organic production. Dissolved oxygen is present at or near saturation throughout the lake during all seasons of the year. (See Eutrophic Lakes.)

OMBROTROPHY Air induced changes in water quality.

OMNIVOROUS Animal which is a first order consumer at some times and a second or higher order consumer at others. (See Herbivorous, Carnivorous.)

OPTIMUM LEVEL The most suitable degree of an environmental factor for the full development of the organism concerned. (See Tolerance Range.)

ORGANISM Any living individual.

OSMOREGULATION The adjustment in the osmotic concentration of solutes in body fluids in organisms to environmental conditions (e.g., when salmon migrate from salt to freshwater).

OVERTURN The period of mixing (turnover) by top to bottom circulation of previously stratified water masses. This phenomenon may occur in spring and/or fall; the result is a uniformity of physical and chemical properties of the water at all depths. (See Thermal Stratification, Chemical Stratification, Spring Overturn, Fall Overturn.)

OXIDATION The loss of electrons by an atom or ion.

OXIDIZING AGENT A substance capable of accepting electrons from another substance and, thereby, being reduced.

OXYGEN DEBT A temporary phenomenon that occurs in an organism when available oxygen is inadequate to supply the respiratory demand. During such a period the metabolic processes result in the accumulation of breakdown products that are not oxidized until sufficient oxygen becomes available.

OXYGEN DEFICIT The difference between observed oxygen concentration and the amount that would theoretically be present at 100% saturation for existing conditions of temperature and pressure.

P

PARASITE An organism that lives on or in a host organism during all or part of its existence. Nourishment is obtained at the expense of the host.

PATHOGEN An organism or virus that causes a disease.

PELAGIC ZONE The open sea, away from the shore. Comparable with the limnetic zone of lakes.

PERCOLATION Infiltration.

PERIODIC DRIFT Drift of bottom organisms at regular or predictable intervals such as diurnal, seasonal, etc. (See Drift Organisms, Catastrophic Drift, Incidental Drift.)

PERIPHYTON (Aufwuchs) Attached microscopic organisms growing on the bottom, or other submersed substrates, in a waterway.

PESTICIDE Any chemical preparation used to kill pests. Include insecticides, herbicides, fungicides, etc.

PESTS (Nuisance Organisms)

pH See Appendix 1: Chemistry.

PHOTOSYNTHESIS The metabolic process by which simple sugars are manufactured from carbon dioxide and water by plant cells using light as an energy source. (See Chlorophyll.)

PHOTIC ZONE (Euphotic Zone)

PHYTOPLANKTON The plants of the plankton. Unattached microscopic plants subject to movement by wave or current action. (See Zooplankton.)

PLANKTON Suspended microorganisms that have relatively low powers of locomotion or that drift in the water subject to the action of waves and currents. (See Benthos, Periphyton, Nekton.)

POND WEEDS (Higher Aquatic Plants)

POOLS Areas of a stream, where the velocity of current is reduced. The reduced velocity provides a favorable habitat for plankton. Silts and other loose materials that settle to the bottom of pools are favorable for burrowing forms of benthos. (See Riffle.)

POPULATION A group of interacting individuals of the same species, area, or community.

POSITIVE ESTUARY Coastal indentures in which there is a measurable dilution of sea water by land drainage; freshwater inflow + precipitation evaporation. (See Inverse Estuary, Neutral Estuary.)

POTAMON ZONE Stream reach at lower elevations characterized by reduced flow, higher temperature, and lower dissolved oxygen levels. (See Rithron Zone.)

ppm (parts per million) A unit of concentration equivalent to the number of milligrams of solute in 1 liter of solution.

PRECIPITATE 1. (noun) A solid which separates from a solution because of some chemical or physical change. 2. (verb) The formation of such a solid.

PREDATOR An animal that kills and consumes other animals. (See Prey.)

PREDOMINANT Those organisms that are of outstanding abundance in a particular community for a given period of time. (See Dominant.)

PREY An animal that is killed and consumed by another animal. (See Predator.)

PRIMARY PRODUCTIVITY The total quantity of protoplasm produced by autotrophic organisms per unit of time in a specified habitat.

PRODUCERS Organisms that synthesize organic material from inorganic substances (e.g., plants). (See Consumers, Reducers.)

PRODUCTION The process of producing organic material; the quantity produced.

PRODUCTIVITY Rate of protoplasm formation or energy utilization by one or more organisms; total quantity of organic material produced within a given period in a specified habitat.

PROFUNDAL ZONE The deep, bottom-water area beyond the depth of effective light penetration. All of the lake floor beneath the hypolimnion.

PROLIFIC	Pertaining to organisms that have a high reproductive potential and normally produce large numbers of young.
PROTOPLASM	The living material in cells of plants and animals.
PUPA	An intermediate, usually quiescent, form following the larval stage in insects, and maintained until metamorphosis to the adult stage. (See Larva.)

Q

QUALITY A term to describe the composite chemical, physical, and biological characteristics of a water with respect to its suitability for a particular use.

QUIESCENT Refers to the temporary cessation of development, movement or other activity. (See Pupa.)

R

RAPIDS Areas of a stream where velocity of current is great enough to keep the bottom clear of all loose materials, thus providing a firm substrate. The surface of the water is disrupted by turbulent currents. This area is occupied largely by specialized benthic or periphytic organisms that can firmly attach or cling to a firm substrate. (See Pools, Riffles.)

RED TIDE A visible red-to-orange coloration of an area of the sea caused by the presence of a bloom of certain plankton. These blooms are often the cause of major fish kills.

REDD A type of fish spawning area associated with flowing water and clean gravel. Fishes that utilize this type of spawning area include trout, salmon, some minnows, etc.

REDUCERS (Decomposers) Those organisms, usually bacteria or fungi, that break down complex organic material into simpler compounds. (See Producers, Consumers.)

REDUCING AGENT A substance capable of releasing electrons to another substance, thereby, being oxidized.

REDUCTION The gain of electrons by an atom or an ion.

REEF A ridge of rocks, sand, soil, or coral projecting from the bottom to or near the surface of the water.

RESPIRATION The complex series of chemical and physical reactions in all living organisms by which the energy and nutrients in foods are made available for use. Oxygen is used and carbon dioxide released during this process. (See Metabolism.)

RIFFLES Fast sections of a stream where shallow water races over stones and gravel. Riffles usually support a wider variety of bottom organisms than other stream sections. Also called rifts. (See Pools, Rapids.)

RITHRON ZONE Stream reach at higher elevations characterized by rapid flow, low temperature, and high dissolved oxygen levels. (See Potamon Zone.)

Appendix 6

ROUGH FISH Those species of fish considered to be of either poor fighting quality when taken on tackle, or of poor eating quality (e.g., carp, gar, suckers, etc.). These fish are considered undesirable in most situations. Most species in the group are more tolerant of widely changing environmental conditions than game fish.

S

SALT MARSH Low area adjacent to the sea that is covered with salt tolerant vegetation and regularly flooded by the high tide; similar inland areas near saline springs or lakes, though not regularly flooded.

SAPROBIC Living on dead or decaying organic matter. (See Scavenger.)

SAPROBICITY The sum of all metabolic processes which are the direct opposite of primary production; can be measured either by the dynamics of metabolism or analysis of community structure.

SAPROBIENSYSTEM European system of classifying organisms according to their response to organic pollution in slow moving streams: 1. Alpha-Mesosaprobic Zone - Area of active decomposition, partly aerobic, partly anaerobic, in a stream heavily polluted with organic wastes; 2. Beta-Mesosaprobic Zone - That reach of a stream that is moderately polluted with organic wastes; 3. Oligosaprobic Zone - That reach of a stream that is slightly polluted with organic wastes and contains the mineralized products of self-purification from organic pollution; but with none of the organic pollution remaining; 4. Polysaprobic Zone - That area of a grossly polluted stream which contains the complex organic wastes that are decomposing primarily by anaerobic processes.

SCAVENGER An organism that consumes decomposing organic matter.

SECONDARY PRODUCTIVITY Total quantity of animal (and other Heterotrophic) protoplasm produced per unit of time in a specified habitat. (See Primary Productivity, Productivity.)

SEDIMENT The material that settles to the bottom of a waterway.

SEEPAGE Any flow of ground water to the surface of the land. This can be in wells, springs, streams or in trickles of water we see in areas such as roadside cuts.

SEICHE Periodic oscillations in the water level of a lake or inland sea. These oscillations take place when a temporary local depression or elevation of the water level occurs.

SENSITIVE ORGANISMS (Intolerant Organisms) Organisms that exhibit a rapid response to environmental changes and are killed, driven out of the area, or as a group are substantially reduced in numbers when their environment is fouled. (See Tolerant Association.)

SESSILE Pertaining to those organisms that are attached to a substrate and not free to move about (e.g., periphyton). (See Free-swimming.)

SESTON All material, both organic and inorganic, suspended in a waterway.

SLOPE The term used to describe the steepness of a hillside. It is often expressed in degrees (of an angle) or in per cent. A ten per cent slope means an increase in altitude of 10 feet for every 100 horizontal feet traveled.

SLUDGE DEPOSITS Accumulations of settled, usually rapidly decomposing, organic material in the aquatic system.

SLUDGEWORMS Aquatic segmented worms (class Oligochaeta) that exhibit marked population increases in waters polluted with decomposable organic wastes. (See Bloodworms.)

SPAWN 1. In aquatic animals, to produce or deposit eggs or sperm. 2. To produce eggs or young. 3. Eggs of fishes and higher aquatic invertebrates.

SPECIES (Both singular and plural) An organism or organisms forming a natural population, or groups of populations, that transmit specific characteristics from parent to offspring. Each species is reproductively isolated from other populations with which they might breed. Hybrids, the results of interbreeding, usually exhibit a loss of fertility.

SPORT FISH (Game Fish)

SPRING OVERTURN A physical phenomenon that may take place in a body of water during the early spring. The sequence of events leading to spring overturn include: (1) melting of ice cover, (2) warming of surface waters, (3) density changes in surface waters producing convection currents from top to bottom, (4) circulation of the total water volume by wind action, and (5) vertical temperature equality. The overturn results in a uniformity of the physical and chemical properties of the entire water mass. (See Fall Overturn, Overturn.)

SPRING TIDE Exceptionally high tide which occurs twice per lunar month when there is a new or full moon, and the earth, sun, and moon are in a straight line. (See Neap Tides.)

STANDARD (Water Quality Standard)

STANDING CROP The quantity of living organisms present in an environment at a selected point in time.

STENO- Prefix denoting a narrow range of tolerance of an organism to a specific environmental factor (e.g., stenothermal refers to temperature; stenohaline refers to salinity; etc.). (See Eury-.)

STIMULUS An influence that causes a response in an organism. (See Taxis.)

STRATIFICATION (Density Stratification) Arrangement of water masses into separate, distinct, horizontal layers as a result of differences in density; may be caused by differences in temperature, dissolved or suspended solids. (See Thermal Stratification, Chemical Stratification.)

SUBLITTORAL ZONE The part of the shore from the lowest water level to the lower boundary of plant growth; transition zone from the littoral to profundal bottom.

SUBMERSED (Submerged Aquatic Plants) Higher aquatic plants that grow beneath the surface of the water (e.g., pondweed, coontails, etc.).

SUBSTRATE The bottom material of a waterway; the base or substance upon which an organism is growing; a substance undergoing oxidation.

SUMMER KILL Complete or partial kill of a fish population in ponds or lakes during the warm months, variously produced by excessively warm water, by a depletion of dissolved oxygen, and by the release of toxic substances from a decaying algal bloom, or by a combination of these factors. (See Winter Kill.)

SUPERSATURATION A condition in which a solution has more solute dissolved than is normally possible under the existing conditions.

SUPRALITTORAL ZONE (Supratidal Zone) The portion of the seashore adjacent to the tidal or spray zone.

SURFACE AQUATIC PLANTS (Floating Aquatic Plants)

SUSPENDED SOLID Any solid substance present in water in an undissolved state, usually contributing directly to turbidity.

SYMBIOSIS Two organisms of different species living in close association, one or both of which may benefit and neither is harmed.

SYNERGISM The joint action of two or more substances is greater than the sum of the action of each of the individual substances (e.g., action of certain combinations of toxicants). (See Antagonism.)

SYNTHESIS The production of a substance by the union of elements or simpler chemical compounds.

SYSTEMATICS (Taxonomy)

T

TARN Small mountain lake or pond.

TAXIS Directed movement by an organism in response to a stimulus (e.g., phototaxis is directed movement in response to a light stimulus; thermotaxis is directed movement in response to heat or cold as a stimulus; etc.).

TAXON (Taxa) Any taxonomic unit or category of organisms (e.g., species, genus, family, order, etc.).

TAXONOMY (Systematics) Organism classification with reference to its relationship in the plant, animal, or protist kingdoms; includes the bases, principles, procedures and rules of classification.

TERRITORY The area which an animal defends against intruders.

THERMAL STRATIFICATION The layering of water masses owing to different densities in response to temperature. The condition of a body of water in which the successive horizontal layers have different temperatures, each layer more or less sharply differentiated from the adjacent ones, the warmest (or the coldest) at the top. (See Overturn.)

THERMOCLINE (Mesolimnion, Metalimnion) The transition zone between the warm epilimnion and cold hypolimnion of stratified bodies of water; temperature change equals or exceeds 1°C for each meter of depth. (See Thermal Stratification.)

THRESHOLD (Critical Level) The maximum or minimum duration or intensity of a stimulus that is required to produce a response in an organism.

TIDAL FLAT The sea bottom, usually wide, flat, muddy and nonproductive, which is exposed at low tide.

TIDAL MARSH A low, flat marshland that is intersected by channels and tidal sloughs, usually covered by high tides; vegetation consists of rushes, grasses, and other salt tolerant plants. (See Salt Marsh.)

TIDAL ZONE (Eulittoral Zone, Intertidal Zone) The area of a shore between the limits of water level fluctuation; the area between the levels of high and low tides.

TIDE The alternate rising and falling of water levels, twice in each lunar day, due to gravitational attraction of the moon and sun in conjunction with the earth's rotational force.

TITRATION The determination of the volume of a solution needed to react with a known volume of sample, usually involving the progressive addition of the solution to the sample until the sample has reacted fully.

TL_m (Median Tolerance Limit)

TOLERANCE Relative capability of an organism to endure an unfavorable environmental factor.

TOLERANCE LIMIT ($TL_{10 \cdots 100}$) The concentration of a substance which some specified portion of an experimental population can endure for a specified period of time with reference to a specified type of response (e.g., TL_{100} means that all test organisms endured the stress for the specified time; TL_{10} means only 10% of the test organisms could tolerate the imposed stress for the specified time). (See Median Tolerance Limit.)

TOLERANCE RANGE The range of one or more environmental conditions within which an organism can function; range between the highest and lowest value of a particular environmental factor in which an organism can live.

TOLERANT ASSOCIATION An association of organisms capable of withstanding adverse conditions within the habitat. This association is often characterized by a reduction in the number of species (from a clean water association) and, in the case of organic pollution, an increase in individuals representing certain species.

TOXICANT A substance that through its chemical or physical action, kills, injures, or impairs an organism; any environmental factor which, when altered, produces a harmful biological effect. (See Pesticide.)

TOXICITY Quality, state, or degree of the harmful effect resulting from alteration of an environment factor.

TOTAL COLIFORM See Appendix 1: Bacteriology.

TRIPTON The dead suspended particulate matter in aquatic habitats; the nonliving portion of the seston. (See Detritus.)

TROPHIC LEVEL One of the parts in a nutritive series in an ecosystem in which a group of organisms in a certain stage in the food chain secures food in the same general manner. The first or lowest trophic level consists of producers (green plants); the second level of herbivores; the third level of secondary carnivores. Most bacteria and fungi are organisms in the reducer (decomposer) trophic level.

TROPHOGENIC REGION The area of a body of water where organic production from mineral substances takes place on the basis of light energy and photosynthetic activity.

TRANSPIRATION The photosynthetic and physiological process by which plants release water into the air in the form of water vapor.

TURBIDITY See Appendix 1: Chemistry.

TURNOVER (Overturn)

U

UBIQUITOUS ORGANISMS Organisms that can tolerate a wide range of environmental conditions or variation; organisms that are so active or numerous as to seem to be present or existent in all types of environments. (See Tolerant Association, Sensitive Organisms.)

UNICELLULAR Refers to an organism that consists of only one cell (e.g., blue-green algae, protozoa, bacteria). These organisms may, however, be filamentous or colonial in form.

V

VERTEBRATES Animals that have an internal skeletal system. (See Invertebrate.)

W

WATER QUALITY CRITERIA "A scientific requirement on which a decision or judgement may be based concerning the suitability of water quality to support a designated use." (See Water Quality Standard.)

WATER QUALITY STANDARD "A plan that is established by governmental authority as a program for water pollution prevention and abatement." (See Water Quality Criteria.)

WATERSHED The area of land delineated by the line separating areas of land, the water from which drains into separate river or stream systems.

WATER TABLE The level of ground water of an area.

WINTER KILL The death of fishes in a body of water during a prolonged period of ice and snow cover; caused by oxygen exhaustion due to respiration and lack of photosynthesis. (See Summer Kill.)

Z

ZONE An area characterized by similar flora or fauna; a belt or area to which certain species are limited.

ZOOPLANKTON The animals of the plankton. Unattached microscopic animals having minimal capability for locomotion.

Appendix 7

Laboratory and/or Field Safety

The items below are arranged according to areas of application; the general comments apply to most areas.

A. General Comments

1. When heating liquids or when working with acids, bases, or other caustic liquids, wear goggles or some other form of eye protection.

2. Show concern for others by not pointing the opening of a container (which you are heating or to which you are adding chemicals) in the direction of fellow workers or their work.

3. Looking directly into containers which are being heated is very dangerous.

4. When such things as strong acids and strong bases are mixed with water or with each other, large amounts of heat are generated. Therefore, use pyrex containers and do not hold them in your hands. Leaving them unattended while they are still hot may cause injury to others.

5. Always pour acid into water, never water into acid.

6. If spillage occurs, turn off open flames and hot plates. Clean up spills immediately. The following procedure should be followed for cleaning up acids, bases, and other caustic substances.

    a. Get paper towels and a large beaker partially filled with water.

    b. Grasp two or three folded paper towels from one end; daub and swab with the other end.

    c. Place wet towels in the beaker.

    d. When the liquid is cleaned up, wipe the area with a damp sponge or several thicknesses of wet paper towels.

    e. Take beaker, wet paper towels, and sponge to sink and rinse with lots of water.

    f. Squeeze wet paper towels and place in waste basket.

7. Acids, bases, caustic substances, and water samples which might be contaminated should not be pipetted by drawing the liquid into the pipette with your mouth. If a rubber bulb is not available, use a burette or a graduated cylinder.

8. Keep volatile liquids (alcohol, ether, petroleum derivatives, etc.) away from open flames.

9. The laboratory is a place of work. Playing around is wasteful of time, equipment, and supplies.

10. For the safety of yourself and others, label all containers which contain solid chemicals, liquids, etc.

   "My uncle was a chemist
   He isn't any more,
   'Cause what he thought was H_2O,
   Was H_2SO_4!"

11. Before leaving the laboratory, check your clothing for spilled substances and thoroughly wash your hands.

B. Bacterial Studies

1. Treat all cultures as if they were pathogenic (disease causing).

2. Plastic and rubber should not be autoclaved.

3. Stay with the autoclave while it is in operation. Turn it off before you leave.

4. Keep work area and equipment sterile. This is necessary from a health standpoint and to prevent contamination of your cultures.

5. Disposal of cultures and resterilization of plastic petri dishes can be accomplished as follows:

  a. Using forceps remove the petri dish covers, and then place covers, dishes, and cultures into a large beaker or pan containing undiluted, liquid household bleach.

  b. After 10 minutes remove the petri dishes, using tongs or a rubber glove, and rinse them well under running water. The wet pads and filter should be put into a plastic bag and discarded.

  c. Immerse the petri dishes and covers in a solution of 70% isopropyl (rubbing) alcohol for 10 minutes.

  d. Remove the petri dishes and covers and stack them on a clean surface. Assemble the dishes and covers. (They are now ready for reuse.)

C. Chemistry

1. Tasting chemicals is dangerous and rarely leads to conclusive results.

2. Should the occasion arise for smelling gases given off by chemical reactions, waft the rising gases to your nose with gentle sweeps of the hand.

3. To avoid sudden dangerous situations from occurring, plan your activity in advance.

4. Always read and reread labels. Particularly note cautions on labels.

5. When connecting rubber and glass apparatus, lubricate the glass with water and assemble with a twisting motion.

6. Be careful when heating or cooling chemicals; be sure the container is designed to be heated. Graduated cylinders and bottles usually are not designed to withstand rapid temperature changes.

D. Field Trips

1. Be sure to take a first aid kit on all field trips.

2. Wear appropriate clothing. Use old sneakers when wading in unknown waters and swamps.

3. If you are going to be using boats in deep water, be sure that everyone has a life jacket or other floatation device.
4. Pair off when working in situations where drowning could occur.
5. Plan for emergency services by obtaining phone numbers of appropriate services in the field area.

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